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(54) Title: HEXAMERIC FUSION PROTEINS AND USES THEREFOR

(57) Abstract

A hexameric fusion protein containing a dimeric binding protein provided with a tailpiece from an IgA antibody is described. This fusion protein is useful in therapeutics and vaccines, but is particularly well suited for applications for which the binding protein from which it is derived is unsatisfactory because of low binding affinity or for applications where multivalency is desired. These applications include diagnostics, binding assays and screening assays.

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HEXAMERIC FUSION PROTEINS AND USES THEREFOR

Related Application

This application claims benefit of U.S. Provisional patent applications numbered 60/019,934 filed June 14, 1996, 60/043,948 filed February 19, 1997 and 60/038,915 filed February 21, 1997.

Background of the Invention

IgM and IgA are the two classes of human antibodies that form homo-oligomeric structures. By far the most extensively studied of these is IgM.

The classical view of IgM structure is as a pentamer in combination with a single copy of a second protein, the J-chain that becomes associated with IgM during its assembly and export. This J-chain can covalently associate with IgM through the formation of a disulfide bond between a cysteine residue in the J chain and a cysteine residue in a short 18 amino acid extension, designated μtp, from the canonical C-terminal constant region of the heavy chain. This cysteine residue appears to be required for the formation of the IgM pentamer in association with the J-chain [A. C. Davis *et al.*, EMBO J., 8(9):2519-2526 (1989)]. However, a hexameric form of IgM, devoid of the J-chain, was described and characterized over two decades ago and has recently been characterized in more detail in terms of its biochemical and potential biological activities [reviewed in Brewer *et al.*, Immunology Today, 15:165-168 (1994)]. The production of oligomeric IgG proteins has been achieved by addition of the 18 amino acid IgM tailpiece segment (utp) to the αtp corresponding C-termini end of the Cγ3 region of the IgG1-4 proteins by DNA recombinant technology [R. I. F. Smith and S. L. Morrison, Biotechnology, 12:683-688 (1994); R. I. F. Smith *et al.*, J. Immunol., 154:2226-2236 (1995)].

Human IgA also has an 18 amino acid tailpiece segment (αtp) which bears some sequence homology to utp. In man, there are two α constant region loci which encode distinct sequences, but the tailpiece regions for the α1 and α2 regions are quite similar, or in some cases reported to be identical [*Sequences of Proteins of Immunological Interest*, fifth edition, EA Kabat *et al.*, Vol. 1, U.S. Department of Health and Human Services, NIH publication no. 91-3242, (1991)]. However, unlike IgM, IgA occurs most frequently as a monomer antibody, similar to the IgG subclasses, or as a dimer antibody plus one molecule of J-chain [Mestecky and Kilian, Methods in Enzymology, 116:37-75 (1985); T. B. Tomasi, Immun.

Today, 13:416-418 (1992)]. Higher oligomers/aggregates of IgA are reported [Mestecky and Kilian, cited above], but these are poorly characterized components in complex mixtures containing other proteins interactive with IgA. Recombinant IgA has been expressed in the presence and absence of theJ chain (Bruggemann *et al.*, J. Exp. Med., 166:1351-1361 (1987); Morton *et al.*, J. Immunol., 151:4743-4752 (1993); Carayannopoulos *et al.*, Proc. Natl Acad Sci. USA, 91:8348-8352 (1994); Terskikh *et al.*, Mol. Immunol., 31:1313-1319 (1994)]. The IgA proteins produced in the absence of the J chain were monomeric or dimeric forms by nonreducing SDS/PAGE and appeared as dimers in solution. In one study (Carayannopoulos *et al.*, above), the co-expression of the J-chain led to formation of disulfide linked IgA dimers together with J chain.

The CD28 receptor, a member of the immunoglobulin superfamily of molecules (IgSF) [A.F. Williams and A.N. Barclay, Annu. Rev. Immunol., 6:381-405 (1988)], is a 44 kDa homodimer glycoprotein expressed on the surface of T-lineage cells including thymocytes and peripheral T cells in the spleen, lymph node and peripheral blood. CD28 interacts with two different counter-receptors CD80 (also known as B7 and B7.1) [P. S. Linsley *et al.*, Proc. Natl. Acad. Sci. USA, 87(13):5031-5035 (1990); G. J. Freeman *et al.*, J. Exp. Med., 174(3):625-631 (1991)] and CD86 (also called B7.2 and B70) [M. Azuma *et al.*, Nature, 366(6450):76-79 (1993); G. J. Freeman *et al.*, J. Exp. Med., 178(6):2185-2192 (1993); G.J. Freeman *et al.*, Science, 262(5135):909-911 (1993)], expressed on antigen presenting cells (APCs), to deliver crucial co-stimulatory signals for sustained activation of T cells, through its association via the cytoplasmic domain with PI3-kinase [F. Pages *et al.*, Nature, 369(6478):327-329 (1994); P. H. Stein *et al.*, Molecul. & Cell. Biol., 14(5):3392-3402 (1994)] and other signalling pathways [K.E. Truitt *et al.*, J. Immunol., 155:4702-4710 (1995); J. A. Nunes *et al.*, J. Biol. Chem., 271(3):1591-1598 (1996); H. Schweider *et al.*, Eur. J. Immunol., 25:1044-1050 (1995)]. Both CD80 [P. S. Linsley *et al.*, J. Exp. Med., 174(3):561-569 (1991)] and CD86 [Azuma *et al.*, cited above; Freeman *et al.*, 1993, cited above ; Freeman *et al.*, 1993, cited above] also recognize CTLA-4 [J.F. Brunet *et al.*, Nature, 328(6127):267-270 (1987)], a homolog of CD28, expressed transiently and at low receptor density on activated $CD8^+$ and $CD4^+$ T cells.

Antagonism of CD28 interactions with the CD80 or CD86 counter-receptors using CTLA4-Ig fusion proteins or antibodies directed against CD80 and CD86 inhibits T cell activation *in vitro*, suppresses humoral and cellular immune responses *in vivo*, inhibits graft rejection and the progression of autoimmune diseases *in vivo* [reviewed in J. A. Bluestone,

Immunity, 2:555-559 (1995); Harlan *et al.*, Clin. Immunol. and Immunopath., 75(2):99-111 (1995)]. Thus, CD28 is a target for development of immunosuppressive agents. To identify small molecule antagonists, a rapid and reproducible assay is desirable for the screening of synthetic compounds, natural products, and peptides. Particularly desirable is a protein based assay which would isolate the receptor and its counter-receptor from interference by other components of cell-based assays, and which is additionally adaptable to automation. The affinity of the interaction of CD28 with both counter receptors is quite low [P. S. Linsley *et al.*, Immunity, 1:793-801 (1994)], with an approximate Kd of 200 nM for the binding of a soluble CD80-Ig fusion protein to an immobilized CD28-Ig fusion protein [P. S. Linsley *et al.*, J. Exp. Med., 173(3):721-730 (1991)]. This low affinity hampers development of a sensitive protein binding assays amenable to screening many compounds.

What is needed is a method for increasing the avidity of binding proteins, particularly those with low affinity, for use in screening and diagnostic assays, therapeutics, and vaccines.

Summary of the Invention

In one aspect, the present invention provides a hexameric fusion protein which provides increased binding activity as compared to the protein from which it is derived and methods of making same. This fusion protein is particularly useful in binding assays and may be readily purified.

The hexameric fusion protein of the invention contains a dimeric binding protein and a tailpiece (αtp) characterized by the activity of the tailpiece from the C-terminus of the heavy chain of an IgA antibody. In one embodiment, the binding protein is a natively dimeric binding protein or a functional fragment thereof. In another embodiment, the binding protein is recombinantly engineered to have a dimeric form. This is preferably achieved by fusion of a protein fragment which contains the extracellular domain of a selected binding protein to an Fc fragment. These binding proteins, when provided with the αtp, assemble into homo- or hetero-hexamers.

In yet another aspect, the present invention provides a polynucleotide sequence encoding a stable hexameric fusion protein of the invention.

In a further aspect, the present invention provides a vector comprising the above-described polynucleotide sequence and a sequence controlling expression of the fusion protein in a selected host cell.

In still another aspect, the present invention provides a recombinant host cell containing the above-described vector.

In a further aspect, the present invention provides methods of producing and purifying a stable hexameric fusion protein by providing a host cell containing the stable hexameric fusion protein of the invention, recovering the stable hexameric fusion protein, and purifying the recovered protein. The strands of the fusion protein are preferably co-produced and assembled in the host cell.

In still a further aspect, the present invention provides a pharmaceutical composition containing a stable hexameric fusion protein or a DNA sequence encoding the stable hexameric fusion protein of the invention and a pharmaceutically acceptable carrier.

In yet another aspect, the present invention provides for screening for ligands to a hexameric fusion protein of the invention. Also provided are assays for inhibitors of hexameric binding protein/ligand interaction.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Brief Description of the Drawings

Fig. 1 is a schematic representation of the hexameric CD80-Igαtp protein of the invention. The regions of the molecule corresponding to the CD80 extracellular domain, the IgG1 hinge, CH2, and CH3 domains, and the αtp segment are indicated. The letter "S" in the diagram indicates the positions of predicted disulfide bonds between cysteine residues.

Fig. 2 is a plasmid map illustrating the expression construct for the CD80-Igαtp protein of the invention. The plasmid is 7,167 base pairs in size. Beginning at residue 1 in a clockwise manner: "cmv pro" is the major late CMV promoter for transcription of the downstream CD80-Igαtp coding sequence; "CD80" encodes the signal peptide and extracellular domain of human CD80; "Fc" encodes the hinge, CH2, and CH3 regions of human IgG1; "αtp" encodes the human αtp segment; "BGH" is the polyadenylation signal region from the bovine growth hormone gene; "betaglobin" is the mouse major b-globin promoter; "dhfr" encodes the mouse dhfr(dihydrofolate reductase) protein; "SV40" is the SV40 early polyadenylation region; and "ori" and "amp" are the bacterial origin of replication and beta lactamase gene, respectively, from the common cloning plasmid pBR322. The corresponding plasmids CD86Fcαtplink and CTLA4Fcαtplink were constructed for the expression of the CD86-Igαtp and CTLA4-Igαtp proteins (see Figs. 5 and 6).

Fig. 3A-3H is the complete DNA sequence of the CD80Fcαtplink plasmid [SEQ ID NO: 1] shown in Fig. 2.

Fig. 4A-4D is the DNA and encoded protein [SEQ ID NOS: 2 and 3] sequences for the CD80-Igαtp region in the vector CD80-Fcαtplink. Bolded regions show restriction sites for reference to Fig. 2 and the initiation codon, mature processing site, hinge region, and C-terminal αtp segment.

Fig. 5A-5B is the DNA and encoded protein sequences [SEQ ID NOS: 4 and 5] for the extracellular domain of CD86 in the vector CD86Fcαtplink. The sequence outside of the Kpn I and Eag I sites is the same as for CD80Fcαtplink (see Figs. 3A-3H and 4A-4D).

Fig. 6A-6C is the DNA and encoded protein sequences [SEQ ID NOS: 6 and 7] for the CMV promoter and the extracellular domain of CTLA-4 in the vector CTLA4-Fcαtplink. The sequence 5' to base 514 and 3' of the Eag I site is the same as for CD80Fcαtplink.

Fig. 7 is a profile for chromatography of CD80-Igαtp on a Superdex 200 column. The first peak eluting at about 45 min is the hexameric protein complex while the second peak migrates at the position observed for monomeric CD80-Ig. The inset shows a coomassie stained pattern for the purified CD80-Igαtp protein on SDS/PAGE under reducing (R) and nonreducing (NR) conditions.

Fig. 8 is a chart showing equilibrium sedimentation (main panel) and sedimentation velocity (inset) analytical centrifugation of the CD80-Igatp protein with a modeled fit to a hexamer/$(\text{hexamer})_2$ equilibrium. The upper graph shows the residuals for the equilibrium sedimentation centrifugation.

Fig. 9 is a line graph illustrating the binding of biotinylated CD80-Igαtp (labeled B7-FcA) to CD28-Ig immobilized at three different concentrations in an ELISA format. Binding was inhibited by the mAb CD28.1 or by CTLA4-Ig.

Fig. 10 is a line graph illustrating the binding of biotinylated CD80-Igαtp, CD86-Igαtp, and CD80-Ig compared to immobilized CD28-Ig in an ELISA format.

Fig. 11 is a line graph illustrating the binding of biotinylated CD80-Igαtp, CD86-Igαtp, and CD80-Ig compared to immobilized CTLA4-Ig in an ELISA format.

Fig. 12 is a line graph illustrating the competition of biotinylated CD80-Igαtp binding to immobilized CD28-Ig (coated at 200 mg/ml) by CD80-Igαtp itself, CD80-Ig, CTLA4-Ig, and CD28.2 MAb.

Fig. 13A is a line graph illustrating the binding of CD80-Igαtp to wild-type and mutant immobilized CD28-muIg2a proteins.

Fig. 13B is a line graph illustrating the binding of CD86-Igαtp to wild-type and mutant immobilized CD28-muIg2a proteins.

Fig. 13C is a line graph illustrating the binding of rabbit polyclonal antisera to wild-type and mutant immobilized CD28-muIg2a proteins.

Fig. 14 is a chart illustrating sequentially the binding of CD80-Ig and CD80-Igαtp to CD28-Ig immobilized on a biosensor chip as measured by surface plasmon resonance.

Fig. 15 is a chart illustrating the binding of CD80-Igαtp and CD86-Igαtp to CD28-Ig immobilized on a biosensor chip as measured by surface plasmon resonance.

Figs. 16A and 16B are line graphs illustrating the binding of CD80-Igαtp and CD86-Igαtp, respectively, to cells expressing human CD28 on their surface in the presence or absence of a CD28 monoclonal antibody that inhibits this interaction.

Fig. 17 is a bar chart illustrating the level of IL-2 production by PCD28.1 cells treated with monomeric and hexameric CD80 (labeled B7.1-Ig and B7.1-IgA, respectively) and CD86 (labeled B7.2-Ig and B7.2-IgA, respectively) Ig fusion proteins. The proteins were used (1) alone in solution, (2) alone immobilized through goat anti-human antibody (GAH), or (3) immobilized in combination with immobilized CD3 mAb. Controls were GAH alone, or with CD3 mAb, and the CD28 IgM mAb 248.23.2. IL-2 levels were determined by CTLL-2 bioassay using known amounts of IL-2 as a standard (inset).

Fig. 18 is a bar chart illustrating the level of IL-2 production by DC27.CD28wt cells treated as described in Fig. 17.

Fig. 19 is a bar chart illustrating IL-2 promoter activity in PCD28.1 cells stimulated as described in Fig.17. IL-2 promoter activity was measured by induction of ß-galactosidase activity which serves as a reporter gene under the control of an IL-2 promoter.

Figs. 20A and 20B are bar graphs respectively showing the induction of the IL-2 promoter, and IL-2 production by CD28 expressing cells incubated with CD80-Igαtp, CD86-Igαtp, or CD80-Ig.

Fig. 20C is a bar graph showing the levels of IL-2 production induced with soluble CD80-Igαtp and CD86-Igαtp in comparison to that induced by immobilized antibody to CD3.

Fig. 21 is a bar chart illustrating inhibition of biotinylated CD80-Igαtp binding to immobilized CD28-Ig by individual compounds in the BM-34 test set. The percent inhibition range is plotted against the number of compounds showing that range of inhibition.

Fig. 22 is a profile for Superose 6 chromatography of the chimeric derivative of the Epo receptor antibody 1C8 (here labeled "anti-EPOr-IgG_1") and the αtp construct of the same antibody (labeled "anti-EPOr-IgG_1αtp") with binding activity to an immobilized EPOr-Ig protein shown in the inset.

Detailed Description of the Invention

The invention provides an hexameric fusion protein useful in therapeutic and immunogenic compositions. The hexameric fusion protein of the invention is particularly well suited for applications for which the binding protein from which it is derived is unsatisfactory because of low binding affinity/avidity and for other applications where multivalency is desired. These applications include diagnostics, binding assays, screening assays and cellular responses based on receptor cross-linking. Also provided are compositions and methods for production and purification of these fusion proteins.

The invention further provides methods of producing stable hexameric fusion proteins, by providing a selected binding protein with an IgA tailpiece (αtp) or a functional equivalent thereof. The inventors have found that addition of the αtp from the natively monomeric or dimeric IgA, surprisingly, provides the resulting fusion protein with the ability to form stable hexamers.

I. Fusion Proteins

As used herein, a hexameric fusion protein of the invention contains a dimeric binding protein which has been provided at its carboxy terminus with a tailpiece (αtp) characterized by having the activity of the tailpiece from the C-terminus of the heavy chain of an IgA antibody. This tailpiece, when attached to each monomer of the dimeric binding protein, provides the resulting fusion protein with the ability to form stable hexamers, i.e., the hexameric fusion proteins of the invention do not undergo any appreciable dissociation in solution (e.g., phosphate buffered saline) at room temperature.

In a particularly preferred embodiment, the fusion proteins of the invention are homo-hexamers. However, where desired, hetero-hexamers comprising two different fusion proteins may be constructed.

The binding proteins useful in the invention include full-length proteins and fragments thereof which are characterized by the binding ability of the full-length protein, i.e., the fragment which has the ability to bind to the counter-receptors or other ligands of the selected binding protein. Such binding proteins may be derived from a protein or protein complex which natively dimerizes for biological activity, or may be genetically engineered as described herein. Examples of suitable natively dimeric binding proteins are those with carboxyl termini situated such that addition of the αtp to the carboxyl terminus of each polypeptide chain, with or without a linker, allows juxtaposition of the αtp chains. One of skill may readily select such native dimeric proteins or dimeric protein complexes, which include, for example, IgG, IgD, or IgE antibodies, Fab fragments, Fab_2 fragments, Ig-Fc fragments, Ig fusion proteins, and the extracellular domains of cell surface proteins such as the α/β chain of a T cell receptor, CD28 and CTLA4, CD8 α/β hetorodimers and α/α homodimers, and the α/β chain of integrin proteins and various cytokine receptors (e.g., IL3, IL5, etc.). These binding proteins are available from a variety of commercial and academic sources. Alternatively, these sequences may be chemically synthesized.

As discussed above, a selected binding protein may be engineered to be dimeric. For example, a protein fragment comprising a binding domain of a selected monomeric binding protein may be attached to an Ig-Fc fragment which forms dimers. Desirably, the binding protein is selected from surface glycoproteins from the immunoglobulin supergene family and their ligands. For example, in a currently preferred embodiment, the binding protein is selected from CTLA-4 (whose extracellular domain can be expressed as a monomer or dimer) and its counter-receptors CD80 and CD86. However, other proteins, including other binding proteins, are known to those of skill in the art and may be used in the construction of a hexameric fusion protein of the invention. Although a currently preferred embodiment of this invention provides hexameric immunoglobulin fusion proteins, which are exemplified herein, this invention is not so limited. For example, a binding protein may be genetically modified to alter its activity. For example, engineered, mutant forms of IL4 have been described that retain high affinity for its receptor but lack normal agonist activity and serve as antagonists of IL-4 mediated function [see, e.g., N. Kruse et al, EMBO J., 11:3237-3244 (1992) and WO96/04388 (Feb. 15, 1996)]. Such a mutant would be useful in a hexameric IL4-Ig fusion protein according to the invention, serving as an antagonist of IL4 function.

The protein fragment used to construct a dimeric binding protein contains at least a fragment of the extracellular domain of the selected binding protein. For functional binding

activity, this extracellular fragment preferably contains the sequences required for binding, which can be readily determined by one of skill in the art. In a preferred embodiment, which makes use of a eukaryotic production system, the protein fragment also contains an export leader sequence which is native to the binding protein selected. However, other export leader sequences which are capable of exporting the protein may be substituted by one of skill in the art. In one exemplary embodiment, where the target is CD28, the protein fragment is the native leader and extracellular domain from CD80 or CD86. The fragments can be obtained from proteins such as CD80 [P. S. Linsley *et al.*, J. Exp. Med., 173(3):721-730 (1991); Truneh *et al.*, Mol. Immunol., 33(3):321-334 (1996); J. E. Ellis *et al.*, J. Immunol., 56:2700-2709 (1996)], and CD86 [P. S. Linsley *et al.*, Immunity, 1:793-801 (1994); J. E. Ellis *et al.*, cited above; P. S. Linsley *et al.*, J. Exp. Med., 174:561-569 (1991)]. In another embodiment, where the target is CD80 or CD86, the protein fragment is the native leader and extracellular domain from CTLA-4 or CD28.

The Fc fragment used in the construction of the hexameric fusion protein may be from any antibody subclass, except IgA. Thus, the Fc fragment may be derived from the IgG, IgD, or IgE subclass. When the Fc fragment is derived from an IgG antibody, any of the human isotypes, i.e., IgG_1, IgG_2, IgG_3, and IgG_4, may be selected. Further, the parental IgG antibody may be mutated to reduce binding to complement or Ig-Fc receptors [see, e.g., A.R. Duncan *et al.*, Nature, 332:563-564 (1988); A. R. Duncan and G. Winter, Nature, 332:738 (1988); M.-L. Alegre *et al.*, J. Immunol., 148:3461-3468 (1992); M-H Tao *et al.*, J. Exp. Med., 178:661-667 (1993); V. Xu et la, J. Biol. Chem., 269:3469-3474 (1994)]. When the Ig-Fc fragment is derived from IgM, it desirably contains the hinge/CH2/CH3/CH4 sequence, but not the naturally occuring 18 amino acid tailpiece (μtp).

Optionally, the C-terminal end of the IgG_1 CH3 domain of the Fc fragment may be modified by conventional techniques to contain a restriction enzyme site for convenient cloning of the tailpiece segments (i.e., the peptide of the invention). Such modifications are described in more detail in the examples below, and are well known to those of skill in the art.

The peptide used to construct the fusion protein of the invention is derived from tailpiece located at the C-terminus of the heavy chain of an IgA antibody. In a preferred embodiment, this peptide is 18 residues in length and is the αtp segment of the human IgA1 heavy chain or a functional equivalent thereof. One particularly suitable peptide is: PTHVNVSVVMAEVDGTCY [SEQ ID NO: 3]. If desired, this peptide may be modified to remove the glycosylation site by changing 1 or 2 amino acids at residues 5-7 (NVS). For

example, the N (asparagine) may be to changed to Q (glutamine) and/or the S (serine) may be changed to A (alanine). Additionally, up to about 4 amino acid residues of the human IgA CH3 domain may be retained. Alternatively, functional equivalents of the human IgA1 αtp may be selected. Suitable functional equivalents include, for example, gorilla IgG1, human IgA2, rabbit IgA, and mouse IgA. Such functional equivalents may also be modified by removal of glycosylation sites. As described herein, this peptide is linked, directly or indirectly, to the binding protein (e.g., the Ig-Fc fragment) and provides the fusion protein of the invention with the ability to assemble into a stable hexamer.

The fusion protein may contain a linker sequence. Optionally, such a linker may be located between the binding protein (e.g., the Ig-Fc fragment) and the αtp peptide. This linker is preferably an amino acid sequence between about 1 and 20 amino acid residues, and more preferably between about 1 and 12 amino acid residues, in length. Other appropriate or desired linkers may be readily selected by one of skill in the art. Although currently less desired, one of skill in the art may substitute other linkers for the preferred amino acid sequence linkers described above.

Three currently preferred embodiments of the fusion proteins of the invention are described herein, CD80-Igαtp, CD86-Igαtp and CTLA4-Igαtp. These proteins are composed of the native leader and extracellular domains of the CD80 (B7.1), the CD86 (B7.2, B70), and the CTLA-4 surface glycoproteins, respectively, linked to the hinge/CH2/CH3 region of the heavy chain of human IgG_1 (Fc fragment) and terminating in a short tail piece segment from human IgA1 (αtp). Another example of a hexameric protein of the invention is an IgG antibody, where the αtp is joined directly to the carboxy terminus of the heavy chain and a light chain is paired with this heavy chain. The αtp hexameric antibody and Ig fusion proteins of the invention are advantageous over IgM antibodies and IgM fusion proteins in that the hexamers of the invention are readily purified on commercially available chromatography supports and are more efficiently expressed.

These constructs may be made using known techniques. A detailed description of the construction of these exemplary fusion proteins of the invention is provided in the examples below.

Briefly, each chain of a dimeric binding protein is selected or constructed. For example, one preferred binding protein is a recombinant immunoglobulin containing the native leader and extracellular domain fused to an Ig-Fc fragment from the selected human IgG antibody. The αtp is added, optionally by introducing a convenient restriction

endonuclease site near the C-terminus of the binding protein (e.g., an Fc region) using silent mutations of the coding sequence and then cloning a synthetic oligonucleotide into this site that encodes the tailpiece segment. The tailpiece segment is matched to that of the human α-1 chain. The tailpiece provides the fusion protein with the ability to form hexamers and the resulting construct is the hexameric fusion protein of the invention. A schematic representation of the predicted hexamer for an exemplary fusion construct of the invention, CD80-Igαtp, is shown in Fig. 1.

Preferably, the fusion proteins of the invention are produced using recombinant techniques. Desirably, the nucleic acid sequences may be fused and the fusion protein expressed in vitro in a suitable host cell. Alternatively, the fusion proteins of the invention are produced by separately expressing, or co-expressing the nucleic acid sequences encoding the protein fragments and αtp fragment of the invention and fusing the expressed products. Suitably, the resulting fusion protein forms hexamers. These production techniques are discussed in more detail below.

II. Polynucleotide Sequences, Expression and Purification

The present invention further encompasses polynucleotide sequences encoding the fusion proteins of the invention. In addition to the DNA coding strand, the nucleic acid sequences of the invention include the DNA (including complementary DNA) sequence representing the non-coding strand and the messenger RNA sequence. Variants of these nucleic acids of the invention include variations due to the degeneracy of the genetic code and are encompassed by this invention. Such variants may be readily identified and/or constructed by one of skill in the art. Further, the polynucleotide sequences may be modified by adding readily assayable tags to facilitate quantitation, where desirable.

To produce recombinant fusion proteins of this invention, the DNA sequences of the invention are inserted into a suitable expression system, preferably a eukaryotic system. Desirably, a recombinant vector is constructed in which the polynucleotide sequence encoding at least one chain of the fusion protein (i.e., the binding protein/αtp) is operably linked to a heterologous expression control sequence permitting expression of the fusion protein of the invention. Numerous types of appropriate expression vectors and host cell systems are known in the art for expression, including, e.g., mammalian, yeast, bacterial, fungal, drosophila, and baculovirus expression.

The transformation of one or more of these vectors into appropriate host cells results in expression of the fusion proteins of the invention. Other appropriate expression vectors, of which numerous types are known in the art, can also be used for this purpose.

Such production methods permit assembly of the hexameric fusion protein of the invention by the host cell. Typically, such methods will provide a homo-hexameric fusion protein. However, in another embodiment, hexameric fusion proteins of mixed specificity may be produced by co-expression of different fusion proteins (i.e., binding protein/αtp). For example, two fusion proteins recognizing non-competing sites on the same molecule can be co-expressed resulting in hexamers that can bind to two sites on the same molecule, resulting in higher binding avidity than for each fusion protein alone or as a homogenous hexamer. Alternatively, the two fusion proteins can bind to two distinct molecules presented on the same, or different surfaces (e.g., expressed on the same or different cells).

Suitable host cells or cell lines for transfection by this method include mammalian cells, such as Human 293 cells, Chinese hamster ovary cells (CHO), the monkey COS-1 cell line, murine L cells or murine 3T3 cells derived from Swiss, Balb-c or NIH mice. Suitable mammalian host cells and methods for transformation, culture, amplification, screening, and product production and purification are known in the art. [See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman *et al.*, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley *et al.*, U. S. Patent 4,419,446]. Another suitable mammalian cell line is the CV-1 cell line.

Other host cells include insect cells, such as *Spodoptera frugipedera* (Sf9) cells. Methods for the construction and transformation of such host cells are well-known. [See, e.g. Miller *et al.*, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein].

Although less preferred, also useful as host cells for the vectors of the present invention are bacterial cells. For example, the various strains of *E. coli* (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas*, other bacilli and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the proteins of the present invention. Other fungal cells may also be employed as expression systems.

Thus, the present invention provides a method for producing a fusion protein of the invention which involves transforming a host cell, preferably a eukaryote, with at least one expression vector containing a recombinant polynucleotide encoding a fusion protein under the control of a transcriptional regulatory sequence, e.g., by conventional means such as transfection or electroporation. The transformed host cell is then cultured under suitable conditions that allow expression of the fusion protein. The expressed and assembled fusion protein is then recovered, isolated, and purified from the culture medium by appropriate means known to one of skill in the art. In a preferred embodiment, the fusion proteins are assembled by the host cell following co-production of one or more of the fusion proteins of the invention. Alternatively, the hexameric fusion protein may be assembled following recovery from the host cell.

Advantageously, the fusion proteins of the invention can be readily purified using conventional techniques. For example, hexameric Ig fusion proteins of the invention may be readily purified on high affinity, high capacity supports based on protein A and protein G. Such resins are commercially available [Pharmacia Inc.; Bioprocessing Ltd.].

Although less preferred, the hexameric fusion protein may be produced in insoluble form. For example, the proteins may be isolated following cell lysis in soluble form, or extracted in guanidine chloride.

III. Pharmaceutical Compositions and Methods of Use Thereof

The fusion proteins of this invention or DNA sequences encoding them may be formulated into pharmaceutical compositions and administered using a therapeutic or immunogenic regimen compatible with the particular formulation. Pharmaceutical compositions within the scope of the present invention include compositions containing a protein of the invention in an effective amount to have the desired physiological effect.

Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble or water-dispersible form, e.g., saline. Alternatively, suspensions of the active compounds may be administered in suitable conventional lipophilic carriers or in liposomes. In still another alternative, adjuvants may be desired, particularly where the composition is to be used as an immunogen.

The compositions may be supplemented by active pharmaceutical ingredients, where desired. Optional antibacterial, antiseptic, and antioxidant agents in the compositions can perform their ordinary functions. The pharmaceutical compositions of the invention may

further contain any of a number of suitable viscosity enhancers, stabilizers, excipients and auxiliaries which facilitate processing of the active compounds into preparations that can be used pharmaceutically. Preferably, these preparations, as well as those preparations discussed below, are designed for parenteral administration. However, compositions designed for oral or rectal administration are also considered to fall within the scope of the present invention.

As used herein, the terms "suitable amount" or "effective amount" means an amount which is effective to treat or prevent the conditions referred to below. A preferred dose of a pharmaceutical composition containing a fusion protein of this invention is generally effective above about 0.1 mg fusion protein of the invention per kg of body weight (mg/kg), and preferably from about 1 mg/kg to about 100 mg/kg. These doses may be administered with a frequency necessary to achieve and maintain satisfactory fusion protein levels. Although a preferred range has been described above, determination of the effective amounts for treatment or prophylaxis of a particular condition may be determined by those of skill in the art.

Particularly, pharmaceutical compositions containing the hexameric antibody/αtp fusion proteins of the invention are useful as antagonists for the 7 transmembrane (7 TMR) class of cell surface receptors, since such receptors are often arrayed in many copies on cell surfaces and the aggregation of such receptors does not lead to intracelluar signalling (agonism) as can occur for many other types of cell surface receptors. For example, administration of a pharmaceutical compositions containing a hexameric antibody/αtp fusion protein of the invention blockades chemokine receptors, a subfamily of the 7 TMR, and inhibits chemotaxis and activation of target cells such as eosinophils. A second example is CTLA4-Igαtp. CTLA4-Ig is a potent inhibitor of CD80 and CD86 driven stimulation of T-cells through their interaction with CD28. In animal models, CTLA4-Ig has shown benefit in several autoimmune diseases and transplantation.

Since the CD80 and CD86 antigens recognized by CTLA4-Ig are arrayed in many copies on the cell surface, an αtp hexameric form of CTLA4-Ig may provide a more potent antagonist than the standard Ig fusion protein. In another embodiment, a pharmaceutical composition of the invention containing Igαtp fusion proteins of the invention may be used for removal of complement components or components of the blood coagulation cascade to retard clotting.

In one aspect, the invention provides a method for antagonizing cell surface CD80- and CD86-mediated stimulation of CD28 positive cells by administering to the cells a hexameric fusion protein CTLA4-Igαtp. This may be performed *in vivo*, by administering a pharmaceutical composition containing this hexameric fusion protein. In another aspect, the invention provides a method for stimulating (agonist activity) CD28+ T cells by administering the CD80- or CD86-hexameric fusion protein to the cells in culture resulting in stimulation of IL-2 production from these cells. These proteins may be used alone, or in combination with other stimulators of T-cells (e.g., antibodies directed against the T cell receptor-CD3 complex.)

In another embodiment, the compositions of the invention containing Ig-Fc-containing fusion proteins are useful for *in vivo* clearance of soluble ligands, in view of the fact that hexamerization of the Fc domain enhances interaction with complement components and Fc receptors. Thus, ligands bound to the hexameric fusion protein of the invention are efficiently cleared from circulation.

The hexameric fusion proteins of the invention can also serve as agonists, particularly in situations where aggregation can induce a desired response. For example, aggregation is essential for signal transduction through many cell surface receptors - either as a consequence of multivalent presentation of the receptor ligand (eg., a counter receptor on a the surface of a second cell) or through changes induced upon ligand binding, or both. An example of signalling through a cell surface receptor induced by cross-linking through recognition of its counter-receptor on a second cell is CD28 recognition by CD80 or CD86.

Thus, the invention further provides a method for stimulating CD28 positive cells by administering to CD28 positive cells CD80-Igαtp and/or CD86-Igαtp. Examples of soluble ligands inducing signal transduction through binding to their receptors are EGF and growth hormone and both result in receptor dimerization. For these receptors, dimerization induced through antibody binding also can lead to activation [Schreiber *et al.*, Proc. Natl. Acad. Sci. USA, 78:7535 (1981), Fuh *et al.*, Science, 256:1677 (1992)]. Hexameric antibodies against such receptors or hexameric ligand-Ig fusion proteins for these receptors are expected to be more efficient stimulators than the standard dimeric antibodies or ligand Ig fusion proteins. For example, the pharmaceutical compositions containing the hexameric antibodies or cytokine-Ig fusion proteins of the invention are useful in inducing signal transduction in receptors for hematopoietic cytokines, such as erythropoietin, thymopoietin and growth stimulatory factor.

Also provided is a method for suppressing CTLA-4 positive cells by administering CD80-Igαtp and/or CD86-Igαtp to CTLA4 positive cells. This may be performed *in vivo*, by administration of a pharmaceutical composition containing the hexameric proteins. Alternatively, the hexameric proteins are added to CTLA4 positive T-cells in culture resulting in inhibition of IL-2 production from these cells.

In yet another aspect, hexameric Ig-fusion proteins of the invention can also serve as enhanced immunogens for the fused protein fragment due to efficient, receptor-mediated updake for antigen processing and presentation or efficient interaction with proteins of the complement system. Enhanced immunogenicity is desirable for the efficient generation of polyclonal and monoclonal antibodies and for therapeutic vaccination. Thus, the invention further provides a method of immunizing using the pharmaceutical composition of the invention.

IV. Assays

The hexameric fusion proteins of the invention are useful in *in vitro* assays for measuring the binding of the fusion protein to a selected ligand and for identifying the native or synthetic ligand for the binding proteins. Such a ligand includes the native ligand or counter-receptor to the binding protein from which the hexameric fusion protein is derived. For example, where the fusion protein is derived from CD80 or CD86, the ligand may be CD28 or CTLA-4. Alternatively, the ligand may be a derivative of the native counter-receptor, a peptide, peptide-like compound, or a chemical compound which interacts with the fusion protein.

The hexameric fusion proteins may be used for in vivo assays, including, for example imaging. See, e.g., S. M. Larson *et al.*, Acta Oncologica, 32(7-8):709-715 (1993); R. DeJager *et al.*, Seminars in Nuclear Medicine, 23(2):165-179 (Apr. 1993).

Alternatively, a fusion protein of the invention may be used to screen for new ligands. The use of the fusion proteins of this invention in such an assay is particularly well suited for identifying cell surface or multivalent ligands.

Suitable assay methods may be readily determined by one of skill in the art. For example, an ELISA format may be utilized in which the selected ligand is immobilized, directly or indirectly (e.g., via an anti-ligand antibody) to a suitable surface.

Where desired, and depending on the assay selected, the hexameric fusion protein may be immobilized on a suitable surface. Such immobilization surfaces are well known.

For example, a wettable inert bead may be used in order to facilitate multivalent interaction with the hexameric fusion proteins of the invention.

Further, the methods of the invention are readily adaptable to combinatorial technology, where multiple molecules are contained on an immobilized support system. Thus, the fusion proteins of the invention permit screening of chemical compound and peptide based libraries where these agents are presented in a multivalent format compatible with more than one subunit of the hexamer. Monomeric interactions of this type are routinely in the mM range and thus may not be readily detected with monomeric proteins. Advantageously, the avidity of the hexameric fusion proteins of the invention permit direct binding.

Typically, the surface containing the immobilized ligand is permitted to come into contact with a solution containing the fusion protein and binding is measured using an appropriate detection system. Suitable detection systems include the streptavidin horse-radish peroxidase conjugate, direct conjugation by a tag, e.g., fluorescein. Other systems are well known to those of skill in the art. This invention is not limited by the detection system used.

The assay methods described herein are also useful in screening for inhibition of the interaction between a hexameric fusion protein of the invention (and thus, the binding protein from which it is derived) and its ligand(s). For example, one may screen for inhibitors of CD80 and CD86 binding to CD28 and CTLA-4. In a preferred method, a solution containing the suspected inhibitors is contacted with an immobilized recombinant CD28 or CTLA-4 protein substantially simultaneously with contacting the immobilized ligand with the solution containing the hexameric CD80- or CD86-Igαtp protein. The solution containing the inhibitors may be obtained from any appropriate source, including, for example, extracts of supernatants from culture of bioorganisms, extracts from organisms collected from natural sources, chemical compounds, and mixtures thereof. In another variation, the inhibitor solution may be added prior to or after addition of the CD80- or CD86-Igαtp proteins to the immobilized CD28 or CTLA-4 protein. Similar methods may be performed using other hexameric fusion proteins of the invention and their respective ligands.

The large size of the Igαtp fusion proteins is also advantageous for biophysical assay methods dependent on diffusion or rotation of the protein target in solution, such as for example, fluorescence polarization, fluorescence correlation spectroscopy and anisotropic analytical methods.

These examples illustrate the preferred methods for preparing and using the fusion proteins of the invention. These examples are illustrative only and do not limit the scope of the invention.

Example 1 - Production and characterization of exemplary αtp Ig fusion proteins

The following describes the production of CD80-Igαtp, CD86-Igαtp, and CTLA4-Igαtp. Further, for comparison, a construct containing the human IgM tailpiece added to the C-terminus of CD80-Ig was also prepared. This construct, designated CD80-Igutp, differs in amino acid sequence from the αtp derivative as follows:

	CH3	Tailpiece	SEQ ID NO:
IgG1	SLSPGK	(none)	9
μtp	SLSTGK	PTLYNVSLVMSDTAGTCY	25 and 10
αtp	SLSAGK	PTHVNVSVVMAEVDGTCY	26 and 11

A. Construction of Recombinant Ig: Binding Protein Fragment/Fc Fusions

The pHbactCd28neo vector for expression of CD28 was previously described [D. Couez *et al.*, Molecul. Immunol., 31(1):47-57 (1994)]. For expression of CD80, the coding sequence was cloned by PCR and inserted into a derivative [Dr. F. Letourneur, NIH] of pCDLSRα296 [Y. Takebe *et al.*, Molecul. & Cell. Biol., 8(1):466-472 (1988)] as described [C. A. Fargeas *et al.*, J. Exp. Med., 182:667-675 (1995)].

The vector COSFcLink [A. Truneh *et al.*, Mol. Immunol., 33(3):321-334 (1996)] was constructed for expression of proteins C-terminally fused to a human IgG1 Fc region under the transcriptional control of the major late promoter of CMV. The dhfr cassette in this vector permits selection for gene amplification in response to methotrexate. The coding sequences for the native leader and extracellular domain peptide of CD28 and CD80 were grafted onto a human IgG1 heavy chain Fc region in the vector COSFcLink, beginning at the start of the hinge region, in a manner similar to that previously described for CD28 and CD80 [P.S. Linsley *et al.*, J. Exp. Med., 174(3):561-569 (1991)]. The Fc region in this vector was derived from the human plasma leukemia cell line ARH-77 [ATCC CRL 1621] and contains a mutation of cysteine to alanine in the upper hinge region (SEQ ID NO: 27 EPKSA, where the mutation is underscored). The CD28 and CD80 sequences were cloned as KpnI - Eag I fragments by PCR from the vectors described above and inserted into the corresponding sites in COSFcLink. The resulting vectors are termed CD28FcLink and

CD80FcLink, respectively. For CD28-Ig, the junction of receptor/Fc fragment (immunoglobulin junction) is SEQ ID NO: 12 --GPSKP/EPKSA-- and the mature processed N-terminal sequence is SEQ ID NO: 13 NKIL --. For CD80-Ig, the immunoglobulin junction is SEQ ID NO: 14 -HFPDq/EPKSA-- and the mature processed N-terminal sequence is VIHV-- (Fig. 4A-4D). The lower case "q" in CD80 represents the substitution of glutamine for the native asparagine.

CD86-Ig, the corresponding binding protein/Fc construct for CD86 containing the native signal peptide of CD86 (B70) [M. Azuma *et al.*, Nature, 366:76-79 (1993)], was constructed using methods essentially identical to those described above. The signal and extracellular sequences were PCR cloned from a plasmid containing the CD86 (B70) coding region that was obtained by reverse transcriptase/PCR cloning from human B-cell RNA based on the sequence described by M. Azuma *et al.* (above). Sequence analysis confirmed identity of this cloned CD86 (B70) region with that of Azuma *et al.* (above). The amino acid sequence at the junction to the Fc region is: SEQ ID NO: 16 --PPPDHepksa-- where capital and lower case letters indicate CD86 and Fc sequences respectively. The mature processed N-terminal sequence is SEQ ID NO: 17 LKIQ -- (Fig. 5A-5B).

CTLA4-Ig, the corresponding binding protein/Fc construct for human CTLA4 containing the native signal peptide of CTLA4 [P. Dariavach *et al.*, Eur J Immunol, 18: 1901-1905 (1988); Harper *et al.*, J Immunol, 147: 1037-1044 (1991)] was constructed in a similar manner. HuC4.32, a pCDM8 plasmid containing the cDNA sequence for human CTLA4 (Harper *et al.*, above) was provided by the laboratory of P. Golstein (Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, 13288 Marseille Cedex 9, France). For PCR cloning of the extracellular domain, the 5' primer was positioned in the pCDM8 vector. [Abberent cloning led to deletion of about 140 bp upstream of the EcoRI site relative to CD80Fclink (compare Fig. 6 with Fig. 3, below).] The amino acid sequence at the junction spanning the end of the CTLA4 extracellular domain and the hinge region is: SEQ ID NO: 18 --EPCPDSDAepksa-- where capital and lower case letters indicate CTLA4 and Fc sequences respectively and the underlined alanine residue indicates its substitution for phenylalanine in the native CTLA4 sequence. The mature processed N-terminal sequence is SEQ ID NO: 19 MHVA-- (Fig. 6A-6C).

Hexameric forms of the CTLA4, CD80 and CD86 recombinant Ig proteins were created by addition of a sequence encoding the 18 amino acid tail piece region of human IgA1

heavy chain to the C-terminus of the CH3 domain in the expression vectors described above. These methods are described in detail below.

B. Construction of Hexameric Fusion Proteins

For convenience, a Hind III site was introduced into the CH3 domain of CD80FcLink [spanning the 3rd base of the codon for Leu441 [EU numbering, E.A. Kabat *et al.*, cited above] through the 2nd base of the codon for L443]. The Hind III site was introduced by standard PCR methods (eg., PCR Protocols: A Guide to Methods and Applications, Innis *et al.*, eds, 1990) using the following oligonucleotides:

5' oligo (positioned in the hinge region of the vector): SEQ ID NO: 20

EagI

cccaaatcggccgacaaaact

3' oligo (spanning the C-terminus of CH3): SEQ ID NO: 21

XbaI HindIII

tcagcgagctctagactacactcatttacccggagacaagcttaggctcttctgcgt

The PCR fragments were isolated by agarose gel electrophoresis and purified on Spin Bind columns (FMC Corp). The fragment was digested with Eag I and Xba I and cloned into similarly digested CD80FcLink vector and colonies were screened for the newly created Hind III site, yielding the vector CD80FcLink-Hd.

To introduce the αtp sequence, a synthetic oligonucleotide linker encoding this sequence was cloned between the newly created Hind III site and the Xba I site in CD80FcLink-Hd. The complementary oligonucleotides for the linker sequence were:

(5') SEQ ID NO: 22 agcttgtctgcgggtaaacccacccatgtcaatgtgtctgttgtcatggc
(Hind III adaptor)

ggaggtggacggcacctgctactgatagt

(5') SEQ ID NO: 23 ctagactatcagtagcaggtgccgtccacctccgccatgacaacagac
(Xba I adaptor)

acattgacatgggtgggtttacccgcagaca

5 mg of each linker was denatured at 70°C for 10 minutes. The reactions were cooled to room temperature for 20 minutes. The concentration of linker was titrated from 50 to 5 ng using 1000 ng of gel purified CD80FcLink-Hd vector, digested with Hind III/Xba I. Several colonies from each ligation condition were screened for the presence of the αtp linker by PCR and confirmed by DNA sequencing.

A schematic representation of the resulting vector, CD80Fcαtplink, is shown in Fig. 2 and the complete DNA sequence is given in Fig. 3A-3H. The vector sequence may differ in some sites from the actual plasmid, but would be function. Introduction of the CD80-Igαtp coding region into other standard mammalian expression vectors (e.g., pBK-CMV from Stratagene. La Jolla, CA) will give suitable results and can be modified appropriately, e.g., by introduction of a dhfr gene, by one of skill in the art.

The vectors for expression of CD86-Igαtp as derived from the corresponding Ig expression vector by replacing the Fc coding region with the Fc-atp region from CD80-Igαtp. The Fc segment of CD86Fclink was excised by cleavage with Eag I (in the hinge region) and Xba I (following the C-terminus of CH3) and replaced with the corresponding fragment of CD80Fcatplink to give the expression vector CD86Fcatplink. The vector for expression of CTLA4-Igαtp was derived by replacing a SpeI-EagI fragment in CD80Fcatplink with the corresponding fragment from CTLA4Fclink to give the expression vector CTLA4Fcatplink. The SpeI site is at base 46 in the CMV promoter region. The sequences of the CD86 and CTLA4 constructs in the region differing from CD80Fcatplink are given in Figs. 5 and 6.

By a similar approach a vector encoding CD28-Igαtp could be prepared starting the CD28Fclink vector described in part A above, or a similar construct encoding an altered version of the CD28 extracellular sequence.

C. Production and purification

The CD28-Ig, CD80-Ig and CD86-Ig proteins were produced in CHO cells and purified as described in A. Truneh et al., Mol Immunol, 33: 321-334 (1996) and in I. Kariv et al., J Immunol, 157: 29-38 (1996). The CTLA4-Ig protein was produced and purified in a similar manner, using the vector construct described above in part A of this section. The Igαtp fusion proteins were shown to be produced upon transfection of the Fcatplink vectors into COS-7 cells following standard procedures for transfection of COS cells (eg., Current Protocols in Molecular Immunology, edited by F.M. Ausubel *et al.* 1988,

John Wiley & Sons, vol 1, section 9.1) and for immunoblot analysis (eg., JR Jackson *et al.*, J. Immunology, 154:3310-3319 (1995)) with rabbit polyclonal anti-sera prepared against various derivatives of the CD80, CD86, and CTLA4 proteins or goat anti-human Fc antibody. The αtp and μtp constructs of CD80-Ig were compared in terms of their efficiency of expression and oligomerization. As determined by SDS/PAGE and immunoblot analysis, the CD80-Igμtp construct did not express as well as the αtp construct of the invention (not shown). The αtp and μtp proteins were purified from the COS cell supernatants by capture on Prosep A (Bioprocessing, Ltd., Consett County Durham, U.K.) and their state of oligomerization examined by analytical size exclusion chromatography on a 3.2 X 30 mm Superose 6 column run on a Smart System HPLC (Pharmacia Biotech, Piscataway NJ). Both proteins showed a similar profile of a dominant large MW species eluting in the molecular weight range of IgM, consistent with formation of a hexameric structure, and a smaller fraction that eluted at the same size as CD80-Ig itself (not shown). However, the fraction of apparent hexamer in the αtp construct was higher (about 80%) than for the μtp construct (about 60%). Both the higher level of expression and the greater efficiency of oligomer formation indicated that the αtp construct of the invention was superior to the μtp derivative. Subsequently, the CD86-Igαtp and the CTLA4-Igαtp proteins were produced in COS cells at about the same level observed for the CD80-Igαtp protein (0.1 - 0.2 ug/ml). The CD80- and CD86-Igαtp proteins were then produced in a CHO cell system (A. Truneh et al., Mol Immunol, 33: 321-334 (1996)) at levels of 5-10 mg/L. This level of production is comparable to other highly expressed proteins (e.g. antibodies) produced in the same manner in this system.

These results indicate that development of standard amplified CHO cell lines with high production levels of hexamer (50 mg/L or greater) is feasible. A procedure for transfection and amplification in CHO cells is described in P. Hensley *et al.*, J. Biol. Chem., 269:23949-23958 (1994)). Briefly, a total of 30 ug of linearized plasmid DNA (e.g. CD80Fcatplink) is electroporated into 1×10^7 cells. The cells are initially selected in nucleoside-free medium in 96 well plates. After three to four weeks, media from growth positive wells is screened for expression - e.g., in an ELISA format using an antibody directed against the Fc region of human IgG1. The highest expressing colonies are expanded and selected in increasing concentrations of methotrexate for amplification of the transfected vectors. If a commercial vector like pBK-CMV (noted above) is used, a dhfr gene should be

introduced into this plasmid or provided on a second co-transfecting plasmid to allow selection of amplification in methotrexate.

The proteins produced in CHO cells were purified by protein A affinity and size exclusion chromatography. For the CD80-Ig hexamer, thirty liters of conditioned medium containing CD80-Igαtp were chromatographed on a Protein A Sepharose Fast Flow column (Pharmacia) at 20 ml/min. The column (5.0 x 11.6 cm; 225 ml) were preequilibrated in 20 mM sodium phosphate, 150 mM NaCl, pH 7.5 (PBS). After loading, the column was washed with 1.8 L of PBS to baseline absorbance. CD80-Igαtp was eluted with 0.1 M sodium citrate, pH 3.0 at 10 ml/min. The eluate was neutralized immediately with 1 M Tris-HCl, pH 8.0. After filtration with a Sterivex GV filter (Millipore) using a 60 ml syringe, CD80-Igαtp was concentrated using an Amicon stirred cell and a YM100 membrane to 1.3 mg/ml. CD80-Igαtp was frozen using a dry ice ethanol bath and stored at -70°C.

To separate hexamer from monomer, 10 ml of the concentrated CD80-Igαtp was chromatographed on a Superdex 200 column (2.6 x 60 cm; Pharmacia) at 2.5 ml/min. The first peak (eluted at about 45 minutes) containing the majority (about 90%) of the 280 nm absorbing material was pooled (20 ml; 0.6 mg/ml), frozen as before and stored at -70 (Figure 7). This material eluted at approximately the position of thyroglobulin (~700,000 Da.) just behind the void volume. A minor peak at about 57 minutes corresponded to "monomer" CD80-Ig. The integrity of the CD80-Igαtp in the peak fractions is shown by the single band observed in coomassie stained SDS/PAGE gel run under reducing conditions (lane R in the inset in Figure 7). The diffuse nature of the band is characteristic of highly glycosylated proteins and is thus expected for CD80-Igαtp which contains 10 consensus N-linked glycosylation sites per polypeptide chain. Under nonreducing conditions, all of the protein migrates as high molecular weight species (lane NR in Figure 7, insert). This dominant fraction migrated as a symmetrical peak at a MW consistent with a hexamer with a lesser amount of a species that migrated at the size observed for the monomeric CD80-Ig protein (i.e., the Ig homodimer). N-terminal amino acid sequence analysis revealed identity to the previous analysis of CD80-Ig and to that described by others [G. J. Freeman *et al.*, 174(3):625-631 (1991)]. The CD86-Igαtp protein was purified in a similar manner. The CTLA4-Igαtp protein was expressed in COS cells, but not further characterized.

D. Protein characterization - Molecular Size

The size exclusion chromatography noted above during purification was consistent with formation of a homogeneous hexameric species containing six CD80-Ig subunits. The size and homogeneity of the CD80-Igαtp protein produced in CHO cells was also investigated by analytical ultracentrifugation. Equilibrium sedimentation data for CD80-Igαtp in PBS, pH 7.4 is shown in Fig. 8, lower panel. The sample was sedimented at 6000 rpm for 87 hours at 25 °C in a Beckman XL-A analytical ultracentrifuge. The weight average molecular weight for a fit to all the data was 1,125,000 +/- 5,000 Da. The expected molecular mass of the hexamer of 864,000, assuming 2000 Da. for each N-linked glycosylation site. The data could also be fitted to a hexamer <-> $(hexamer)_2$ model with a K_d of ~ 2 x 10^{-7} M. The curves in the lower panel are for the fitted distribution of hexamer and $(hexamer)_2$. The sum of these two curves fits the observed data well. Inclusion of terms for a monomer (131 kDa) did not improve the fit. The distribution of residuals (fitted-observed data) for the fit of the monomer dimer model to the data is shown in the upper panel of Fig 8. The residuals are small and random, indicating a good fit. For a description of the analysis see W. Chan *et al.*, Folding and Design, 1(2): 77-89 (1996). The lower panel inset shows g(s*) analysis of velocity sedimentation data of the protein taken in the absorption mode. Data was collected at 30,000 rpm at 22 °C. The data could be fitted to two species, one of 19.4 S and one of 26.7 S which could be the hexamer and $(hexamer)_2$ species. For g(s*) data analysis, see W. F. Stafford, Current Opinion in Biotechnology,8(1): 14-24 (1997).

The size and extent of covalent association of the CD80- and CD86-Igαtp proteins were examined by SDS/PAGE. Under reducing conditions all of the protein migrated in a diffuse band at about the same size as the corresponding standard Ig constructs, as shown for the CD80-Igαtp protein in the inset in Figure 7. Under nonreducing conditions in a 4% gel, the Igαtp constructs migrated as very diffuse bands in the size range of IgM with little material co-migrating with the corresponding Ig constructs at about 150,000 Da (not shown). These results indicate that most of the individual polypeptide chains in the Igαtp proteins are covalently joined through cystine bonds, consistent with the described disulfide bond formation among the cysteine residues in the μ tailpiece segment of IgM [A. C. Davis et al., EMBO J., 8(9): 2519-2526 (1989)]. The diffuse nature of the high molecular weight

bands may reflect incomplete disulfide bond formation but also is expected since a hexamer form of CD80- or CD86-Igαtp would contain 120 potential N-linked glycosylation sites.

E. Protein Characterization - Binding properties

In several assay formats the hexameric CD80- and CD86-Igαtp proteins were distinguished from the corresponding standard Ig fusion proteins by their markedly higher binding avidity to CD28 when it was presented in a multivalent array.

1) Binding to immobilized CD28-Ig in an ELISA format

For this assay format, the CD80-Igαtp protein was biotinylated for simplicity of assay and for ease of detection since the CD28 protein absorbed to the plate wells was also a human Ig fusion construct. Biotinylation was carried out essentially as described in Avidin-Biotin Chemistry: A handbook, M. D. Savage *et al.*, Pierce Chemical Company (1992). In several preparations of the protein, the molar ratio of biotin/CD80-Ig monomer was about 10:1. All steps of the assay after coating were carried out at room temperature.

The wells of 96 well microtiter plates (Immunlon 4, Dynatech Laboratories) were coated with CD28-Ig (1, 2, or 4 μg/ml) in 100 μl/well of 0.1 M sodium bicarbonate, pH 9.4 and incubated overnite @ 4°C. The wells were washed with PBS (phosphate buffered saline) and blocked with 0.5% gelatin in PBS for 1 hour. Following an additional PBS wash, biotinylated CD80-Igαtp was serially diluted in PBS containing 1 mg/ml BSA, 0.05% Tween directly in the wells in a final volume of 0.1 ml and incubated for 1 hour. The wells were washed with PBS and bound CD80-Igαtp protein was measured by the addition of 0.1 ml of strepavidin-HRP (streptavidin conjugated with horseradish peroxidase (Southern Biotech)) at a 1:2000 dilution for 1 hour,followed by washing and color development with 100 μl ABTS substrate (Kierkegaard and Perry Laboratories Inc., Maryland) and measurement of absorbance at 405 nm. In some cases the color reactions were arrested by addition of 100 μl of 1% SDS prior to measurement of absorbance. A plot of CD80-Igαtp binding versus concentration of added protein is shown in Fig. 9. In this figure, "CD28-Fc", "CTLA4-Fc", and "B7-FcA" denote CD28-Ig, CTLA4-Ig, and CD80-Igαtp, respectively. These curves (Fig. 9) indicate that concentration dependent binding of biotinylated CD80-Igαtp was inhibited by simultaneous addition of the CD28.1 MAb (a murine MAb to human CD28 that inhibits binding of CD80 to CD28; Nunes *et al.*, Int. Immunol., 5:311-315 (1993)) or CTLA4-Ig protein (here labeled as CTLA4-Fc). Under the same conditions, biotinylated CD80-Ig itself showed little binding and only at much higher concentrations (Figure 10). In the same format biotinylated CD86-Igαtp also showed good binding to CD28-Ig (Fig

10). All three biotinylated proteins showed good binding to immobilized CTLA4-Ig (Fig. 11), as expected because of the higher affinity of this interaction [P. S. Linsley et. al., Immunity 1: 793-801 (1994), and see part 4 of this example below], and the rank order of binding was the same as observed with immobilized CD28-Ig.

The specificity of the binding reaction was demonstrated by the expected hierarchical competition of binding with (1) CTLA4-Ig, (2) CD28.2 [Nunes *et al.*, 1993, cited above], a murine MAb to human CD28 that inhibits binding of CD80 to CD28, (3) unlabeled CD80- and CD86-Igαtp proteins, (4) and the expected much weaker inhibition by the monomeric CD80-Ig fusion protein. One example is shown in Fig. 12. Briefly, microtiter wells were coated with 2 μg/ml CD28-Ig and biotinylated CD80-Igαtp was added at a concentration of 50 μg/ml, followed immediately by the indicated amounts of unlabeled CD80-Igαtp (B7FcA), CD80-Ig (B7Ig), CTLA4-Ig, or the MAb CD28.2. At 50 μg/ml, the biotinylated CD80-Igαtp gives about 50% saturation of OD_{405} (see Fig. 9). CD80-Ig was much less efficient than CD80-Igαtp in blocking binding, consistent with the expected lower affinity/avidity of the CD80-Ig protein for the immobilized CD28-Ig protein. The controls gave the expected results - the CD28.2 MAb blocked the binding site on CD28 and similarly, CTLA4-Ig blocked the binding sites on CD80-Igαtp.

Other assay formats are possible. A second example utilizes a CD28-*mu*Ig fusion protein constructed in a manner analogous to CD28-Ig except that the Ig region was derived from mouse Ig2a instead of human IgG1. More particularly, the protein was expressed using the vector CosCD28mFc2aLink, which is comparable to the CosCD28FcLink vector (described above), except that the human IgG1-Fc region was replaced with that of mouse IgG2a beginning at the Eag I site in the hinge sequence [described in I. Kariv *et al.*, J. Immunol., 157:29-38 (1996)]. The amino acid sequence in the resulting hybrid hinge region is as follows: SEQ ID NO: 24 --GPSKPepksag**IKP**--, where capital letters correspond to the end of CD28 sequence, lower case letters are residues from the human IgG_1 hinge region, underlined lower case letters are a 2 residue substitution introduced to create an Eag I site, and bold capital letters indicate the beginning of murine IgG2a hinge region.

The CD28-*mu*Ig protein was indirectly immobilized in wells using goat anti-mouse Fc antibody and then CD80-Igαtp binding was carried out similarly to that described above. More specifically, CD28-*mu*Ig proteins containing wild-type or mutant CD28 sequences and, at equal concentrations, were captured on goat anti-mouse IgG antibody coated 96 well plates. The plates were washed with 1x PBS, blocked with 0.5% gelatin-PBS for 1 hour, and

then incubated with either biotinylated CD80- or CD86-Igαtp for 45 min. The plates were washed and Igαtp fusion protein was quantitatedas described above. . This assay was used to examine the effects of mutations in CD28 on binding to CD80 and CD86, as illustrated in Figs. 13A and 13B (I. Kariv *et al.*, J. Immunol., 157:29-38 (1996)). Each of the mutant CD28-muIg2a proteins was captured on the goat anti-mouse IgG coated wells and the binding of biotinylated CD80 - Igαtp (Fig. 13A) or CD86 - Igαtp (Fig. 13B) was measured. Equivalent capture of each of the CD28-muIg2a proteins was verified by the comparable binding of polyclonal rabbit CD28 antisera to each of the proteins (Fig. 13C).

2) Binding to immobilized CD28-Ig in a biosensor assay format

The binding of CD80-Igαtp or CD80-Ig to immobilized CD28 were compared by surface plasmon resonance analysis using a BIAcore instrument, following procedures similar to that described for other proteins [K. Johanson et. al., J. Biol. Chem, 270: 9459-9471 (1995), and references therein].

For comparison of CD80-Ig and CD80-Igαtp, approximately 4000 RU of CD28-Ig were immobilized onto a BIAcore CM5 sensor surface (BIAcore, Piscataway, NJ) by covalent attachment to the surface through its amines. Covalent attachment was achieved by firstly activating the surface with a 1:1 mixture of 0.1 M solution of N-hydroxysuccinamide and 0.1 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. A solution of CD28-Ig 50 ug/ml in 0.01 M sodium acetate pH 4.7 was then passed over the surface. Unreacted N-hydroxysuccinamide esters were then deactivated with 1 M ethanolamine pH 8.5. The surface was equilibrated with running buffer composed of 20 mM HEPES 150 mM NaCl, pH 7.2, 3 mM EDTA and 0.005% Tween 20. The CD80-Ig and CD80-Igatp (20 ug/ml) diluted in running buffer were injected over the surface (60 ul) with a flowrate of 10 ul/min. The results show that CD80-Ig dissociates very rapidly from the CD28-Ig coated surface, whereas the rate of dissociation for CD80-Igαtp is about three orders of magnitude slower (Figure 14).

For comparison of CD80-Igαtp and CD86-Igαtp binding with immobilized CD28-Ig, solutions of CD80-Igαtp and CD86-Igαtp (5 ug/ml) were prepared in running buffer described above. Sample solutions were injected (60 ul) at 10 ul/min. Between samples, the surface was regenerated with a 30 ul injection of Gentle elution buffer (Pierce Chemicals, Rockville, ILL). The results show that like CD80-Igαtp above, CD86-Igαtp dissociates slowly from the CD28-Ig surface (Figure 15). The off-rate for CD86-Igαtp

appears higher than that for the CD80 construct, consistent with the weaker binding of this protein in the ELISA (part 1, above) and cell binding (part 3, below) formats and the lower intrinsic affinity measured by calorimetry (part 4, below).

3) Binding to cells expressing cell-surface CD28

By flow cytometry, both CD80- and CD86-Igαtp show specific binding to CD28 positive cells (Figs. 16A and 16B), whereas no binding is observed with CD80- or CD86-Ig themselves (not shown). Non-adherent CD28 expressing cells (PCD28.1.s2.1) were used for this assay format. PCD28.1.S2.1 cells were created by transfection of PE30.2 cells [D. Emilie *et al.*, Eur. J.. Immunol., 19:1619-1624 (1989)] with a vector for expression of human CD28 [D. Couez *et al.*, Molecular Immunology, 31:47-57 (1994)]. All incubations were carried out on ice. Unlabelled CD80- and CD86-Igαtp or CD80- and CD86-Ig were incubated with the cells in binding buffer consisting of PBS w/o Ca+2/Mg+2, 0.2% bovine serum albumin and 0.1% sodium azide. After washing twice in binding buffer, bound fusion proteins were detected with a 1:2000 dilution of a goat anti-human polyclonal antibody labeled with FITC (Flourescein isothiocyanate, Southern Biotech). Following two additional washes in binding buffer, the cells were resuspended in binding buffer and analyzed on a FACSort analyzer (Becton-Dickinson) using a 488nm laser. Low non-specific binding was shown by incubating the cells with a 200 fold excess of a CD28 monoclonal antibody 28.1 prior to ("block" curves, Figure C), or at the same time as ("competition" curves, Figs. 16A and 16B), or by addition of CTLA4-Ig with the CD80 and CD86 fusion proteins (not shown).

4) Binding to CD28-Ig and CTLA4-Ig in solution

The solution binding properties of the hexameric and standard Ig fusion proteins of CD80 and CD86 were compared using isothermal titration calorimetry, essentially as described previously for other proteins [K. Johanson et. al., J. Biol. Chem, 270: 9459-9471 (1995), and references therein]. The binding to CTLA4-Ig is summarized in the following table:

Table I

Direct comparison of CTLA4-Ig binding properties of CD80 and CD86 Ig fusion proteins as measured by titration calorimetry at 2 temperatures

Construct	Experimental Temperature	K_d, nM at Temperature	Molar Ratio (CTLA4-Ig per construct)	ΔH, kcal/mol	K_d nM at 37°C
CD80-Ig	37°C	5.4	0.72	-32 ± 2	5
CD80-Igαtp	37°C	5.9	4.02	-36 ± 2	6
CD86-Ig	37°C	38	0.80	-33 ± 3	40
CD86-Igαtp	37°C	20	2.72	-37 ± 4	20
CD80-Ig	44°C	4.9	0.60	-36 ± 4	2
CD80-Igαtp	44°C	8.1	3.79	-38 ± 3	2
CD86-Ig	44°C	71	0.79	-34 ± 4	22
CD86-Igαtp	44°C	38	2.83	-41 ± 4	9

The error in Kd's is about a factor of 2 and the error in molar binding ratio's is 10-20%. Kd values at 37°C were either measured directly at 37°C or were corrected for temperature differences using the van't Hoff equation, as described in M. L. Doyle et. al., J. Mol. Recognition , 9: 65-74 (1996). Concentrations were defined by absorbance at 280 nm using the following: A) molecular masses of 90,059 (CTLA4-Ig), 127,000 (CD80-Ig), 810,000 (CD80-Igαtp), 140,000 (CD86-Ig), and 890,000 (CD86-Igαtp) and B) calculated extinction coefficients of 1.22 (CTLA4-Ig), 1.10 (CD80-Ig and CD80-Igαtp), and 1.03 (CD86-Ig and CD86-Igαtp). The molecular weights for CTLA4-Ig, CD80-Ig, and CD86-Ig were determined by mass spectral analysis. The molecular masses of CD80-Igαtp and CD86-Igαtp were estimated as 6x the mass of the respective Ig proteins plus 40,000 Da. contributed by the twelve tailpiece segments.

Direct comparison of the CTLA4-Ig binding to CD80-Ig, CD80-Igαtp, CD86-Ig, and CD86-Igαtp constructs in solution phase by isothermal titration calorimetry

d monstrates several features. First, the affinities of the Ig versus Ig-αtp constructs are equivalent in solution. This suggests that, as expected, solution binding affinities of the αtp constructs do not benefit from avidity effects like they do in ELISA and cell binding assays. Second, the enthalpy changes which accompany the molecular interactions of the Ig and Igαtp constructs are also the same and support the view that the molecular details of the interactions are the same. Third, the titration equivalence points for CTLA4-Ig binding to the CD80 and CD86 Ig versus Igαtp constructs indicate that all these reagents were ≥50% active during the calorimetry assay. With regard to this latter point, comparison of the Igαtp and Ig constructs shows a ratio of about 6 for CD80, indicating about equivalent binding activity for the CD80 domains in both constructs. The lower ratio for the corresponding CD80-Igαtp protein indicates some loss of activity in this preparation.

Interactions of CD28-Ig with either CD80- or CD86-Ig were not detected in solution by calorimetry, suggesting an affinity of interaction weaker than 1 uM. This lower affinity for CD28 than for CTLA4 is in agreement with other reports [P. S. Linsley et. al., Immunity 1: 793-801 (1994)]. CD28-Ig also did not show detectable binding to CD80- or CD86-Igαtp, which is consistent with the solution affinities of the αtp constructs not benefiting from avidity effects.

Example 2 - Demonstration of agonist activity for the CD80- and CD86 Igαtp protein

A. CTLL-2 bioassay for detection of IL-2 levels

The CD80- and CD86-Igαtp proteins were compared to the corresponding CD80 and CD86-Ig proteins to determine their ability to stimulate cells expressing human CD28 using two murine T-cell hybridoma cell lines expressing human CD28, PCD28.1.s2.1 and DCL27CD28wt.s2. The PCD28.1.s2.1 cell line was described in Example 1, part 3. The DCL27CD28wt.s2 cell line was created by transfection of the DC27 cell line [F. Pages *et al.*, Nature, 369:327-329 (1994); F. Pages *et al.*, J. Biol. Chem., 271(16):9403-9409 (1996)] with the same CD28 expression vector used for the PCD28.1.s2.1 cells [D. Couez *et al.*, Molecular Immunology, 31:47-57 (1994)]. These cell lines were examined for their ability to produce IL-2 in response to activation with CD80- and CD86-Ig in comparison with the corresponding Igαtp fusion proteins. 96-well plates were coated with or without a CD3 antibody together with the CD80 and CD86 fusion proteins. This was accomplished by first incubating the plates with a previously determined suboptimal concentration of hamster anti-human CD3 antibody (MAb 145-2C11, Boerhinger-Mannheim Biochemicals) for two

hours at room temperature (RT) or with just buffer alone, washing the plates with PBS, adding goat anti-human Ig heavy chain (GAH-IgHc, Sigma Chemical Co.) for an additional two hours at RT, washing again and coating with different concentrations of the CD80 or CD86 fusion protein for 16-18 hours at 4°C, washing again, and finally blocking for 30 min. with 0.2% BSA-PBS. T cells ($1x10^5$/well) were added in 150 µl medium into duplicate wells. For comparison, the soluble fusion proteins and the 248.23.2 CD28 MAb (IgM) [A. Morretta, University of Genova, Italy] were added to non-coated wells. T cells were incubated in the wells for 24 hours at 37°C, and supernatants were collected and evaluated for IL-2 levels in a standard CTLL-2 bioassay [S.M. Gillis *et al.*, J. Immunol., 120:2027 (1978)]. Briefly, $1x10^4$ IL-2 dependent CTLL-2 cells (ATCC)/well in 75 µl medium were added to an equal volume of test supernatant and incubated for 24 hours at 37°C. The cells were pulsed with 10 µl of 5 mg/ml MTT (Sigma Chemical Co.) for 4 hours, and lysed with 100 ml 10% SDS/0.01N HCl solution for 14-16 hours. OD_{570} readings were converted into ng/ml of IL-2 based on a standard curve generated by treating cells with known concentrations of IL-2.

In all assays, the CD80- and CD86-Igαtp proteins were more efficient stimulators of the CD28 T-cells than the corresponding monomeric Ig constructs (Figs. 17 and 18). The soluble hexameric proteins induced IL-2 production in the absence of CD3 crosslinking (GAH), whereas under the same conditions, no activity was observed with CD80- or CD86-Ig themselves. A similar level of IL-2 induction was observed with the oligomeric CD28 IgM antibody 248.23.2. Cross-linking of the hexameric CD80 and CD86 proteins with GAH antibody increased the IL-2 response relative to the absence of cross-linker, but still did not give a response for the monomeric Ig constructs. In the presence of CD3 antibody, the differences between the hexameric and monomeric Ig fusion proteins were minimal, being about 2-fold or less.

B. Fluorescein di-b-D-galactosidase (FDG) assay for detection of IL-2 promoter activity

A second assay for agonist activity measured induction of IL-2 promoter activity, rather than production of IL-2 protein. The PCD28.1.S2.1 cells described above also contain *lacZ* fused to the IL-2 promoter. Thus, the PCD28.1.S2.1 cell line provides a convenient system for measuring IL-2 promoter activity upon CD28-mediated T cell simulation. T cells were activated as described above for the CTLL-2 assay, spun down, resuspended in 50 µl of media + 50 µl of PBS, lysed with 10 µl of 20% Triton X-100, and

supplemented with 25 µl of 10 mM FDG (Molecular Probes), a fluorogenic substrate for b-galactosidase. Hydrolysis of FDG first yields fluorescein monogalactoside (FMG) and then the highly fluorescent product fluorescein. Cell lysates were incubated with FDG for 60 min., and the levels of fluorescence were measured by Fluoroscan (MTX Lab Systems, Inc).

The results of these assays (Fig. 19) were similar to those described above for IL-2 production. The primary difference was that low levels of IL-2 promoter activity were observed for the monomeric Ig proteins.

C. Stimulation of CD28 cells by CD80- and CD86-Igαtp proteins in solution

In the above examples (parts A and B), the Ig and Igαtp proteins showed the greatest activity when captured on the surface of the microtiter well.. However, the CD80- and CD86-Igαtp proteins were also able to stimulate CD28 cells when added directly to the cells in solution, whereas no response was observed with the corresponding standard Ig fusion proteins. CD80-and CD86-Igαtp showed a dose dependent stimulation of IL-2 promoter activity (Fig 20A) and IL-2 production (Fig 20B) when added to PC28.1.s2.1 cells. In contrast, no stimulation was observed with CD80-Ig (Figs. 20A and 20B) or CD86-Ig (not shown). IL-2 promoter activity and IL-2 levels were measured similarly to that described in parts A and B above, except that proliferation of the reader CTLL-2 cells was measured by ^{3}H-thymidine incorporation. The level of response at near saturation levels of CD80- and CD86-Igαtp proteins (1 ug/ml) was comparable to that observed for stimulation through cross-linking of CD3 with immobilized CD3 antibody (Fig 20C). The specificity of the response to CD80- and CD86-Igαtp was confirmed by complete blockade with the addition of CTLA4-Ig (not shown).

In summary, the results from these assays show that the CD80- and CD86- Igαtp proteins have agonist activity under conditions where little or no activity was observed for the corresponding monomeric Ig proteins.

Example 3 - Compound screen assay for identifying small molecule antagonists of the interaction between CD28 and CD80

An ELISA format was used to identify small molecule antagonists of CD80 and CD86 binding to CD28 by screening a large bank of chemical compounds and natural products. The assay was carried out as in the format described in Example 1, part E.1, except that immediately following addition of the biotinylated CD80-Igαtp (222 ng/ml in a volume of 90 µl), dilutions of test compound were added (10 µl). The compounds were

dissolved at 100x assay concentration in dimethyl sulfoxide (DMSO) and subsequently diluted in 50%DMSO/50% H_2O to a 10X working stock. The assay was not sensitive (<10% alteration of signal) to DMSO at concentrations of 5% or less.

Results from one test assay are summarized in Fig. 21 and Table II. The BM-34 test set consists of 968 compounds in two formats - as individual compounds and as 88 multimixes with 11 individual compounds in each multimix sample. Both BM-34 formats were assayed (at a concentration of 200 µg/ml for each multimix sample and 20 µg/ml for individual compounds) for inhibition of biotinylated CD80-Igαtp binding to immobilized CD28-Ig in 96 well plates. Results for setting a 70% or 85% cutoff for inhibition are shown in Table II. In Fig. 21, the percent inhibition range is plotted against the number of compounds showing the indicated range of inhibition. The low percentage of compounds showing activity in the 80-90 % range of inhibition makes this a suitable threshold for rapid screening.

Table II

CD80-Igαtp Screen Assay Results

BM-34 Test Compound Set

Result	70% Cutoff	85% Cutoff
Hits on Multimix plates	7	1
+ Multimix Samples with + Compound	5/7	1/1
- Multimix Samples with + Compound	8	0

As illustrated in this table, eight of the mixes gave 70% or greater inhibition. Deconvolution by assay of the individual compounds from these 8 mixes at 20 µg/ml confirmed that there was a compound with comparable activity. The two other mixes that failed to confirm had one or more compounds with activity very close to the 70% inhibition

observed in the original multimix assay. Selecting a higher cutoff of 85% gave only one multimix hit and that was confirmed in the assay of individual compounds. Further evidence of reproducibility and selectivity was that only eight compounds from mixes below the 70% cutoff showed > 70% inhibition when assayed individually. Selectivity was further increased by reducing the concentration of the multimix samples to 100 μg/ml and the individual compounds to 10 μg/ml. This corresponds to about a 30 μM concentration for the compounds since their average MW is 300-400 daltons. At 100 μg/ml, multimix samples showed a desired shift to lower average inhibition (90% of the mixtures gave 60% or less inhibition) while retaining an acceptable hit rate at a high level of inhibition (4% of the mixtures giving 70% or greater inhibition). Through the use of this assay, small molecule inhibitors of the interaction of CD80 with CD28 can be identified.

Example 4 - αtp-mediated oligomerization of a mouse/human IgG1 chimeric antibody.

To examine the generalization of αtp-mediated hexamer formation of the Fc region of human IgG, the αtp segment was introduced into a chimeric antibody containing heavy and light chain variable regions from the mouse monoclonal antibody 1C8 and the human kappa and IgG1 constant regions. 1C8 is directed against the human EPO (erythropoeitin) receptor. The αtp sequence was introduced onto the heavy chain of the antibody by replacing the Eco RI/Sac II fragment of CD80Fcαtplink with the Eco RI/Sac II fragment of EpoR(CH)IgG1-PCN, a vector containing the heavy chain of the chimeric 1C8 antibody, to give the vector EpoR(CH)Fcαtplink. In both vectors, Eco RI cleaves between the CMV promoter and the start of the N-terminal signal sequences and Sac II cleaves at a conserved site in constant region 2 of the human heavy chain.

Test samples of the hexameric mAb were produced in COS-7 cells upon co-transfection of EpoR(CH)Fcαtplink and a vector for expression of the light chimeric light chain, following procedures described above in Example 1, part C. Initially, 5 T150 flasks were co-transfected with the two vectors and 300 ml of conditioned media were collected. The hexameric antibody was purified by affinity chromatography on Protein A. Purity was about 90% as determined by coomassie staining of the sample as analyzed by reducing SDS/PAGE. Under nonreducing conditions on SDS/PAGE, the antibody migrated in the size range of IgM (not shown).

The sample was further characterized by analytical size exclusion chromatography on a 3.2 X 30 mm Superose 6 column run on a Smart System HPLC (Pharmacia Biotech,

Piscataway NJ). The major peak (Fig. 22) corresponds to binding activity, as monitored in an ELISA using a recombinant human EPO receptor Ig fusion protein (EPOr-Ig), and eluted at a size consistent with hexamer formation (anti-EPOr-$IgG_1\alpha tp$). The parental chimeric antibody (anti-EPOr-IgG_1) elutes substantially later from the column and is represented in the figure by the dashed lines.

These results indicate that addition of the αtp segment to the human IgG1 constant region leads to formation of hexameric antibody.

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7167 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GACGTCGACG GATCGGGAGA TCGGGGATCG ATCCGTCGAC GTACGACTAG TTATTAATAG 60

TAATCAATTA CGGGGTCATT AGTTCATAGC CCATATATGG AGTTCCGCGT TACATAACTT 120

ACGGTAAATG GCCCGCCTGG CTGACCGCCC AACGACCCCC GCCCATTGAC GTCAATAATG 180

ACGTATGTTC CCATAGTAAC GCCAATAGGG ACTTTCCATT GACGTCAATG GGTGGACTAT 240

TTACGGTAAA CTGCCCACTT GGCAGTACAT CAAGTGTATC ATATGCCAAG TACGCCCCCT 300

ATTGACGTCA ATGACGGTAA ATGGCCCGCC TGGCATTATG CCCAGTACAT GACCTTATGG 360

GACTTTCCTA CTTGGCAGTA CATCTACGTA TTAGTCATCG CTATTACCAT GGTGATGCGG 420

TTTTGGCAGT ACATCAATGG GCGTGGATAG CGGTTTGACT CACGGGGATT TCCAAGTCTC 480

CACCCCATTG ACGTCAATGG GAGTTTGTTT TGGCACCAAA ATCAACGGGA CTTTCCAAAA 540

TGTCGTAACA ACTCCGCCCC ATTGACGCAA ATGGGCGGTA GGCGTGTACG GTGGGAGGTC 600

TATATAAGCA GAGCTGGGTA CGTGAACCGT CAGATCGCCT GGAGACGCCA TCGAATTCGG 660

TTACCTGCAG ATATCAAGCT AATTCGGTAC CGGGCCCCCC TCGAGCCTGA AGCCATGGGC 720

CACACACGGA GGCAGGGAAC ATCACCATCC AAGTGTCCAT ACCTCAATTT CTTTCAGCTC 780

TTGGTGCTGG CTGGTCTTTC TCACTTCTGT TCAGGTGTTA TCCACGTGAC CAAGGAAGTG 840

AAAGAAGTGG CAACGCTGTC CTGTGGTCAC AATGTTTCTG TTGAAGAGCT GGCACAAACT 900

CGCATCTACT GGCAAAAGGA GAAGAAAATG GTGCTGACTA TGATGTCTGG GGACATGAAT 960

ATATGGCCCG AGTACAAGAA CCGGACCATC TTTGATATCA CTAATAACCT CTCCATTGTG 1020

ATCCTGGCTC TGCGCCCATC TGACGAGGGC ACATACGAGT GTGTTGTTCT GAAGTATGAA 1080

AAAGACGCTT TCAAGCGGGA ACACCTGGCT GAAGTGACGT TATCAGTCAA AGCTGACTTC 1140

CCTACACCTA GTATATCTGA CTTTGAAATT CCAACTTCTA ATATTAGAAG GATAATTTGC 1200

TCAACCTCTG GAGGTTTTCC AGAGCCTCAC CTCTCCTGGT TGGAAAATGG AGAAGAATTA 1260

AATGCCATCA ACACAACAGT TTCCCAAGAT CCTGAAACTG AGCTCTATGC TGTTAGCAGC 1320

AAACTGGATT TCAATATGAC AACCAACCAC AGCTTCATGT GTCTCATCAA GTATGGACAT 1380

TTAAGAGTGA ATCAGACCTT CAACTGGAAT ACAACCAAGC AAGAGCATTT TCCTGATCAG 1440

GAGCCCAAAT CGGCCGACAA AACTCACACA TGCCCACCGT GCCCAGCACC TGAACTCCTG 1500

GGGGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ACACCCTCAT GATCTCCCGG 1560

ACCCCTGAGG TCACATGCGT GGTGGTGGAC GTGAGCCACG AAGACCCTGA GGTCAAGTTC 1620

AACTGGTACG TGGACGGCGT GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG 1680

TACAACAGCA CGTACCGGGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT 1740

GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC 1800

ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA CCACAGGTGT ACACCCTGCC CCCATCCCGG 1860

GATGAGCTGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTATCCCAGC 1920

GACATCGCCG TGGAGTGGGA GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACGCCT 1980

CCCGTGCTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC 2040

AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC 2100

TACACGCAGA AGAGCCTAAG CTTGTCTGCG GGTAAACCCA CCCATGTCAA TGTGTCTGTT 2160

GTCATGGCGG AGGTGGACGG CACCTGCTAC TGATAGTCTA GAGCTCGCTG ATCAGCCTCG 2220

ACTGTGCCTT CTAGTTGCCA GCCATCTGTT GTTTGCCCCT CCCCCGTGCC TTCCTTGACC 2280

CTGGAAGGTG CCACTCCCAC TGTCCTTTCC TAATAAAATG AGGAAATTGC ATCGCATTGT 2340

CTGAGTAGGT GTCATTCTAT TCTGGGGGGT GGGGTGGGGC AGGACAGCAA GGGGGAGGAT 2400

TGGGAAGACA ATAGCAGGCA TGCTGGGGAT GCGGTGGGCT CTATGGAACC AGCTGGGGCT 2460

CGAGGGGGGA TCTCCCGATC CCCAGCTTTG CTTCTCAATT TCTTATTTGC ATAATGAGAA 2520

AAAAAGGAAA ATTAATTTTA ACACCAATTC AGTAGTTGAT TGAGCAAATG CGTTGCCAAA 2580

AAGGATGCTT TAGAGACAGT GTTCTCTGCA CAGATAAGGA CAAACATTAT TCAGAGGGAG 2640

TACCCAGAGC TGAGACTCCT AAGCCAGTGA GTGGCACAGC ATTCTAGGGA GAAATATGCT 2700

TGTCATCACC GAAGCCTGAT TCCGTAGAGC CACACCTTGG TAAGGGCCAA TCTGCTCACA 2760

CAGGATAGAG AGGGCAGGAG CCAGGGCAGA GCATATAAGG TGAGGTAGGA TCAGTTGCTC 2820

CTCACATTTG CTTCTGACAT AGTTGTGTTG GGAGCTTGGA TAGCTTGGAC AGCTCAGGGC 2880

TGCGATTTCG CGCCAAACTT GACGGCAATC CTAGCGTGAA GGCTGGTAGG ATTTTATCCC 2940

CGCTGCCATC ATGGTTCGAC CATTGAACTG CATCGTCGCC GTGTCCCAAA ATATGGGGAT 3000

TGGCAAGAAC GGAGACCTAC CCTGGCCTCC GCTCAGGAAC GAGTTCAAGT ACTTCCAAAG 3060

AATGACCACA ACCTCTTCAG TGGAAGGTAA ACAGAATCTG GTGATTATGG GTAGGAAAAC 3120

CTGGTTCTCC ATTCCTGAGA AGAATCGACC TTTAAAGGAC AGAATTAATA TAGTTCTCAG 3180

TAGAGAACTC AAAGAACCAC CACGAGGAGC TCATTTTCTT GCCAAAAGTT TGGATGATGC 3240

CTTAAGACTT ATTGAACAAC CGGAATTGGC AAGTAAAGTA GACATGGTTT GGATAGTCGG 3300

AGGCAGTTCT GTTTACCAGG AAGCCATGAA TCAACCAGGC CACCTTAGAC TCTTTGTGAC 3360

AAGGATCATG CAGGAATTTG AAAGTGACAC GTTTTTCCCA GAAATTGATT TGGGGAAATA 3420

TAAACTTCTC CCAGAATACC CAGGCGTCCT CTCTGAGGTC CAGGAGGAAA AAGGCATCAA 3480

GTATAAGTTT GAAGTCTACG AGAAGAAAGA CTAACAGGAA GATGCTTTCA AGTTCTCTGC 3540

TCCCCTCCTA AAGCTATGCA TTTTTATAAG ACCATGCTAG CTTGAACTTG TTTATTGCAG 3600

CTTATAATGG TTACAAATAA AGCAATAGCA TCACAAATTT CACAAATAAA GCATTTTTTT 3660

CACTGCATTC TAGTTGTGGT TTGTCCAAAC TCATCAATGT ATCTTATCAT GTCTGGATCA 3720

ACGATAGCTT ATCTGTGGGC GATGCCAAGC ACCTGGATGC TGTTGGTTTC CTGCTACTGA 3780

TTTAGAAGCC ATTTGCCCCC TGAGTGGGGC TTGGGAGCAC TAACTTTCTC TTTCAAAGGA 3840

AGCAATGCAG AAAGAAAAGC ATACAAAGTA TAAGCTGCCA TGTAATAATG GAAGAAGATA 3900

AGGTTGTATG AATTAGATTT ACATACTTCT GAATTGAAAC TAAACACCTT TAAATTCTTA 3960

AATATATAAC ACATTTCATA TGAAAGTATT TTACATAAGT AACTCAGATA CATAGAAAAC 4020

AAAGCTAATG ATAGGTGTCC CTAAAAGTTC ATTTATTAAT TCTACAAATG ATGAGCTGGC 4080

CATCAAAATT CCAGCTCAAT TCTTCAACGA ATTAGAAAGA GCAATCTGCA AACTCATCTG 4140

GAATAACAAA AAACCTAGGA TAGCAAAAAC TCTTCTCAAG GATAAAAGAA CCTCTGGTGG 4200

AATCACCATG CCTGACCTAA AGCTGTACTA CAGAGCAATT GTGATAAAAA CTGCATGGTA 4260

CTGATATAGA AACGGACAAG TAGACCAATG GAATAGAACC CACACACCTA TGGTCACTTG 4320

ATCTTCAACA AGAGAGCTAA AACCATCCAC TGGAAAAAAG ACAGCATTTT CAACAAATGG 4380

TGCTGGCACA ACTGGTGGTT ATCATGGAGA AGAATGTGAA TTGATCCATT CCAATCTCCT 4440

TGTACTAAGG TCAAATCTAA GTGGATCAAG GAACTCCACA TAAAACCAGA GACACTGAAA 4500

CTTATAGAGG AGAAAGTGGG GAAAAGCCTC GAAGATATGG GCACAGGGGA AAAATTCCTG 4560

AATAGAACAG CAATGGCTTG TGCTGTAAGA TCGAGAATTG ACAAATGGGA CCTCATGAAA 4620

CTCCAAAGCT ATCGGATCAA TTCCTCCAAA AAAGCCTCCT CACTACTTCT GGAATAGCTC 4680

AGAGGCCGAG GCGGCCTCGG CCTCTGCATA AATAAAAAAA ATTAGTCAGC CATGCATGGG 4740

GCGGAGAATG GGCGGAACTG GGCGGAGTTA GGGGCGGGAT GGGCGGAGTT AGGGGCGGGA 4800

CTATGGTTGC TGACTAATTG AGATGCATGC TTTGCATACT TCTGCCTGCT GGGGAGCCTG 4860

GGGACTTTCC ACACCTGGTT GCTGACTAAT TGAGATGCAT GCTTTGCATA CTTCTGCCTG 4920

CTGGGGAGCC TGGGGACTTT CCACACCCTA ACTGACACAC ATTCCACAGA ATTAATTCCC 4980

GATCCCGTCG ACCTCGAGAG CTTGGCGTAA TCATGGTCAT AGCTGTTTCC TGTGTGAAAT 5040

TGTTATCCGC TCACAATTCC ACACAACATA CGAGCCGGAA GCATAAAGTG TAAAGCCTGG 5100

GGTGCCTAAT GAGTGAGCTA ACTCACATTA ATTGCGTTGC GCTCACTGCC CGCTTTCCAG 5160

TCGGGAAACC TGTCGTGCCA GCTGCATTAA TGAATCGGCC AACGCGCGGG GAGAGGCGGT 5220

TTGCGTATTG GGCGCTCTTC CGCTTCCTCG CTCACTGACT CGCTGCGCTC GGTCGTTCGG 5280

CTGCGGCGAG CGGTATCAGC TCACTCAAAG GCGGTAATAC GGTTATCCAC AGAATCAGGG 5340

GATAACGCAG GAAAGAACAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG 5400

GCCGCGTTGC TGGCGTTTTT CCATAGGCTC CGCCCCCCTG ACGAGCATCA CAAAAATCGA 5460

CGCTCAAGTC AGAGGTGGCG AAACCCGACA GGACTATAAA GATACCAGGC GTTTCCCCCT 5520

GGAAGCTCCC TCGTGCGCTC TCCTGTTCCG ACCCTGCCGC TTACCGGATA CCTGTCCGCC 5580

TTTCTCCCTT CGGGAAGCGT GGCGCTTTCT CAATGCTCAC GCTGTAGGTA TCTCAGTTCG 5640

GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT GTGCACGAAC CCCCCGTTCA GCCCGACCGC 5700

TGCGCCTTAT CCGGTAACTA TCGTCTTGAG TCCAACCCGG TAAGACACGA CTTATCGCCA 5760

CTGGCAGCAG CCACTGGTAA CAGGATTAGC AGAGCGAGGT ATGTAGGCGG TGCTACAGAG 5820

TTCTTGAAGT GGTGGCCTAA CTACGGCTAC ACTAGAAGGA CAGTATTTGG TATCTGCGCT 5880

CTGCTGAAGC CAGTTACCTT CGGAAAAAGA GTTGGTAGCT CTTGATCCGG CAAACAAACC 5940

ACCGCTGGTA GCGGTGGTTT TTTTGTTTGC AAGCAGCAGA TTACGCGCAG AAAAAAAGGA 6000

TCTCAAGAAG ATCCTTTGAT CTTTTCTACG GGGTCTGACG CTCAGTGGAA CGAAAACTCA 6060

CGTTAAGGGA TTTTGGTCAT GAGATTATCA AAAAGGATCT TCACCTAGAT CCTTTTAAAT 6120

TAAAAATGAA GTTTTAAATC AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC 6180

CAATGCTTAA TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT 6240

GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC TGGCCCCAGT 6300

GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG ATTTATCAGC AATAAACCAG 6360

CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT CCTGCAACTT TATCCGCCTC CATCCAGTCT 6420

ATTAATTGTT GCCGGGAAGC TAGAGTAAGT AGTTCGCCAG TTAATAGTTT GCGCAACGTT 6480

GTTGCCATTG CTACAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC TTCATTCAGC 6540

TCCGGTTCCC AACGATCAAG GCGAGTTACA TGATCCCCCA TGTTGTGCAA AAAAGCGGTT 6600

AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG CCGCAGTGTT ATCACTCATG 6660

GTTATGGCAG CACTGCATAA TTCTCTTACT GTCATGCCAT CCGTAAGATG CTTTTCTGTG 6720

ACTGGTGAGT ACTCAACCAA GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT 6780

TGCCCGGCGT CAATACGGGA TAATACCGCG CCACATAGCA GAACTTTAAA AGTGCTCATC 6840

ATTGGAAAAC GTTCTTCGGG GCGAAAACTC TCAAGGATCT TACCGCTGTT GAGATCCAGT 6900

TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT CTTTTACTTT CACCAGCGTT 6960

TCTGGGTGAG CAAAAACAGG AAGGCAAAAT GCCGCAAAAA AGGGAATAAG GGCGACACGG 7020

AAATGTTGAA TACTCATACT CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT 7080

TGTCTCATGA GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG 7140

CGCACATTTC CCCGAAAAGT GCCACCT 7167

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1560 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 63..1538

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAATTCGGTT ACCTGCAGAT ATCAAGCTAA TTCGGTACCG GGCCCCCCTC GAGCCTGAAG 60

CC ATG GGC CAC ACA CGG AGG CAG GGA ACA TCA CCA TCC AAG TGT CCA 107
Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser Lys Cys Pro
1 5 10 15

TAC CTC AAT TTC TTT CAG CTC TTG GTG CTG GCT GGT CTT TCT CAC TTC 155
Tyr Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu Ser His Phe
20 25 30

TGT TCA GGT GTT ATC CAC GTG ACC AAG GAA GTG AAA GAA GTG GCA ACG 203
Cys Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr
35 40 45

CTG TCC TGT GGT CAC AAT GTT TCT GTT GAA GAG CTG GCA CAA ACT CGC 251
Leu Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg
50 55 60

ATC TAC TGG CAA AAG GAG AAG AAA ATG GTG CTG ACT ATG ATG TCT GGG 299
Ile Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly
65 70 75

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GAC ATG AAT ATA TGG CCC GAG TAC AAG AAC CGG ACC ATC TTT GAT ATC      347
Asp Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile
 80                  85                  90                  95

ACT AAT AAC CTC TCC ATT GTG ATC CTG GCT CTG CGC CCA TCT GAC GAG      395
Thr Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu
                100                 105                 110

GGC ACA TAC GAG TGT GTT GTT CTG AAG TAT GAA AAA GAC GCT TTC AAG      443
Gly Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys
            115                 120                 125

CGG GAA CAC CTG GCT GAA GTG ACG TTA TCA GTC AAA GCT GAC TTC CCT      491
Arg Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro
        130                 135                 140

ACA CCT AGT ATA TCT GAC TTT GAA ATT CCA ACT TCT AAT ATT AGA AGG      539
Thr Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg
    145                 150                 155

ATA ATT TGC TCA ACC TCT GGA GGT TTT CCA GAG CCT CAC CTC TCC TGG      587
Ile Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp
160                 165                 170                 175

TTG GAA AAT GGA GAA GAA TTA AAT GCC ATC AAC ACA ACA GTT TCC CAA      635
Leu Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln
                180                 185                 190

GAT CCT GAA ACT GAG CTC TAT GCT GTT AGC AGC AAA CTG GAT TTC AAT      683
Asp Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn
            195                 200                 205

ATG ACA ACC AAC CAC AGC TTC ATG TGT CTC ATC AAG TAT GGA CAT TTA      731
Met Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu
        210                 215                 220

AGA GTG AAT CAG ACC TTC AAC TGG AAT ACA ACC AAG CAA GAG CAT TTT      779
Arg Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe
    225                 230                 235

CCT GAT CAG GAG CCC AAA TCG GCC GAC AAA ACT CAC ACA TGC CCA CCG      827
Pro Asp Gln Glu Pro Lys Ser Ala Asp Lys Thr His Thr Cys Pro Pro
240                 245                 250                 255
```

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TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC      875
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
                260                 265                 270

CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA      923
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
            275                 280                 285

TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC      971
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
        290                 295                 300

TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG     1019
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
    305                 310                 315

GAG GAG CAG TAC AAC AGC ACG TAC CGG GTG GTC AGC GTC CTC ACC GTC     1067
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
320                 325                 330                 335

CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC     1115
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
                340                 345                 350

AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA     1163
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
            355                 360                 365

GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT     1211
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
        370                 375                 380

GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC     1259
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
    385                 390                 395

TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG     1307
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
400                 405                 410                 415

AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC     1355
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
                420                 425                 430
```

```
TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG      1403
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
            435                 440                 445

AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC      1451
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
        450                 455                 460

ACG CAG AAG AGC CTA AGC TTG TCT GCG GGT AAA CCC ACC CAT GTC AAT      1499
Thr Gln Lys Ser Leu Ser Leu Ser Ala Gly Lys Pro Thr His Val Asn
    465                 470                 475

GTG TCT GTT GTC ATG GCG GAG GTG GAC GGC ACC TGC TAC TGATAGTCTA       1548
Val Ser Val Val Met Ala Glu Val Asp Gly Thr Cys Tyr
480                 485                 490

GAGCTCGCTG AT                                                       1560
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 492 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser Lys Cys Pro Tyr
 1               5                  10                  15

Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu Ser His Phe Cys
            20                  25                  30

Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu
        35                  40                  45

Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile
    50                  55                  60

Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp
65                  70                  75                  80
```

```
Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr
                85                  90                  95

Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly
            100                 105                 110

Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg
        115                 120                 125

Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr
    130                 135                 140

Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile
145                 150                 155                 160

Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu
                165                 170                 175

Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp
            180                 185                 190

Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met
        195                 200                 205

Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg
    210                 215                 220

Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro
225                 230                 235                 240

Asp Gln Glu Pro Lys Ser Ala Asp Lys Thr His Thr Cys Pro Pro Cys
                245                 250                 255

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
            260                 265                 270

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
        275                 280                 285

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
    290                 295                 300

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
305                 310                 315                 320
```

```
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
                325                 330                 335

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
            340                 345                 350

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
        355                 360                 365

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
    370                 375                 380

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
385                 390                 395                 400

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
                405                 410                 415

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
            420                 425                 430

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
        435                 440                 445

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
    450                 455                 460

Gln Lys Ser Leu Ser Leu Ser Ala Gly Lys Pro Thr His Val Asn Val
465                 470                 475                 480

Ser Val Val Met Ala Glu Val Asp Gly Thr Cys Tyr
                485                 490
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 831 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 52..831

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
TCGGTTACCT GCAGATATCA AGCTAATTCG GTACCAGCAG AAGCAGCCAA A ATG GAT       57
                                                          Met Asp
                                                            1

CCC CAG TGC ACT ATG GGA CTG AGT AAC ATT CTC TTT GTG ATG GCC TTC       105
Pro Gln Cys Thr Met Gly Leu Ser Asn Ile Leu Phe Val Met Ala Phe
          5                  10                  15

CTG CTC TCT GGT GCT GCT CCT CTG AAG ATT CAA GCT TAT TTC AAT GAG       153
Leu Leu Ser Gly Ala Ala Pro Leu Lys Ile Gln Ala Tyr Phe Asn Glu
     20                  25                  30

ACT GCA GAC CTG CCA TGC CAA TTT GCA AAC TCT CAA AAC CAA AGC CTG       201
Thr Ala Asp Leu Pro Cys Gln Phe Ala Asn Ser Gln Asn Gln Ser Leu
 35                  40                  45                  50

AGT GAG CTA GTA GTA TTT TGG CAG GAC CAG GAA AAC TTG GTT CTG AAT       249
Ser Glu Leu Val Val Phe Trp Gln Asp Gln Glu Asn Leu Val Leu Asn
                 55                  60                  65

GAG GTA TAC TTA GGC AAA GAG AAA TTT GAC AGT GTT CAT TCC AAG TAT       297
Glu Val Tyr Leu Gly Lys Glu Lys Phe Asp Ser Val His Ser Lys Tyr
             70                  75                  80

ATG GGC CGC ACA AGT TTT GAT TCG GAC AGT TGG ACC CTG AGA CTT CAC       345
Met Gly Arg Thr Ser Phe Asp Ser Asp Ser Trp Thr Leu Arg Leu His
         85                  90                  95

AAT CTT CAG ATC AAG GAC AAG GGC TTG TAT CAA TGT ATC ATC CAT CAC       393
Asn Leu Gln Ile Lys Asp Lys Gly Leu Tyr Gln Cys Ile Ile His His
    100                 105                 110

AAA AAG CCC ACA GGA ATG ATT CGC ATC CAC CAG ATG AAT TCT GAA CTG       441
Lys Lys Pro Thr Gly Met Ile Arg Ile His Gln Met Asn Ser Glu Leu
115                 120                 125                 130
```

```
TCA GTG CTT GCT AAC TTC AGT CAA CCT GAA ATA GTA CCA ATT TCT AAT      489
Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Val Pro Ile Ser Asn
                135                 140                 145

ATA ACA GAA AAT GTG TAC ATA AAT TTG ACC TGC TCA TCT ATA CAC GGT      537
Ile Thr Glu Asn Val Tyr Ile Asn Leu Thr Cys Ser Ser Ile His Gly
            150                 155                 160

TAC CCA GAA CCT AAG AAG ATG AGT GTT TTG CTA AGA ACC AAG AAT TCA      585
Tyr Pro Glu Pro Lys Lys Met Ser Val Leu Leu Arg Thr Lys Asn Ser
        165                 170                 175

ACT ATC GAG TAT GAT GGT ATT ATG CAG AAA TCT CAA GAT AAT GTC ACA      633
Thr Ile Glu Tyr Asp Gly Ile Met Gln Lys Ser Gln Asp Asn Val Thr
    180                 185                 190

GAA CTG TAC GAC GTT TCC ATC AGC TTG TCT GTT TCA TTC CCT GAT GTT      681
Glu Leu Tyr Asp Val Ser Ile Ser Leu Ser Val Ser Phe Pro Asp Val
195                 200                 205                 210

ACG AGC AAT ATG ACC ATC TTC TGT ATT CTG GAA ACT GAC AAG ACG CGG      729
Thr Ser Asn Met Thr Ile Phe Cys Ile Leu Glu Thr Asp Lys Thr Arg
                215                 220                 225

CTT TTA TCT TCA CCT TTC TCT ATA GAG CTT GAG GAC CCT CAG CCT CCC      777
Leu Leu Ser Ser Pro Phe Ser Ile Glu Leu Glu Asp Pro Gln Pro Pro
            230                 235                 240

CCA GAC CAC GAG CCC AAA TCG GCC GAC AAA ACT CAC ACA TGC CCA CCG      825
Pro Asp His Glu Pro Lys Ser Ala Asp Lys Thr His Thr Cys Pro Pro
        245                 250                 255

TGC CCA                                                             831
Cys Pro
    260
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Asp Pro Gln Cys Thr Met Gly Leu Ser Asn Ile Leu Phe Val Met
 1               5                  10                  15

Ala Phe Leu Leu Ser Gly Ala Ala Pro Leu Lys Ile Gln Ala Tyr Phe
            20                  25                  30

Asn Glu Thr Ala Asp Leu Pro Cys Gln Phe Ala Asn Ser Gln Asn Gln
        35                  40                  45

Ser Leu Ser Glu Leu Val Val Phe Trp Gln Asp Gln Glu Asn Leu Val
    50                  55                  60

Leu Asn Glu Val Tyr Leu Gly Lys Glu Lys Phe Asp Ser Val His Ser
65                  70                  75                  80

Lys Tyr Met Gly Arg Thr Ser Phe Asp Ser Asp Ser Trp Thr Leu Arg
                85                  90                  95

Leu His Asn Leu Gln Ile Lys Asp Lys Gly Leu Tyr Gln Cys Ile Ile
            100                 105                 110

His His Lys Lys Pro Thr Gly Met Ile Arg Ile His Gln Met Asn Ser
        115                 120                 125

Glu Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Val Pro Ile
    130                 135                 140

Ser Asn Ile Thr Glu Asn Val Tyr Ile Asn Leu Thr Cys Ser Ser Ile
145                 150                 155                 160

His Gly Tyr Pro Glu Pro Lys Lys Met Ser Val Leu Leu Arg Thr Lys
                165                 170                 175

Asn Ser Thr Ile Glu Tyr Asp Gly Ile Met Gln Lys Ser Gln Asp Asn
            180                 185                 190

Val Thr Glu Leu Tyr Asp Val Ser Ile Ser Leu Ser Val Ser Phe Pro
        195                 200                 205

Asp Val Thr Ser Asn Met Thr Ile Phe Cys Ile Leu Glu Thr Asp Lys
    210                 215                 220
```

Thr Arg Leu Leu Ser Ser Pro Phe Ser Ile Glu Leu Glu Asp Pro Gln
225 230 235 240

Pro Pro Pro Asp His Glu Pro Lys Ser Ala Asp Lys Thr His Thr Cys
245 250 255

Pro Pro Cys Pro
260

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1104 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 601..1104

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GACGTCGACG GATCGGGAGA TCGGGGATCG ATCCGTCGAC GTACGACTAG TTATTAATAG 60

TAATCAATTA CGGGGTCATT AGTTCATAGC CCATATATGG AGTTCCGCGT TACATAACTT 120

ACGGTAAATG GCCCGCCTGG CTGACCGCCC AACGACCCCC GCCCATTGAC GTCAATAATG 180

ACGTATGTTC CCATAGTAAC GCCAATAGGG ACTTTCCATT GACGTCAATG GGTGGACTAT 240

TTACGGTAAA CTGCCCACTT GGCAGTACAT CAAGTGTATC ATATGCCAAG TACGCCCCCT 300

ATTGACGTCA ATGACGGTAA ATGGCCCGCC TGGCATTATG CCCAGTACAT GACCTTATGG 360

GACTTTCCTA CTTGGCAGTA CATCTACGTA TTAGTCATCG CTATTACCAT GGTGATGCGG 420

TTTTGGCAGT ACATCAATGG GCGTGGATAG CGGTTTGACT CACGGGGATT TCCAAGTCTC 480

CACCCCATTG ACGTCAATGG GAGTTTGTTT TGGCGACTCA CTATAGGAGT TCCCAAGCTT 540

```
CTAGAGATCC CTCGAGATCC ATTGTGCTCT AAAGGACCTG AACACCGCTC CCATAAAGCC      600

ATG GCT TGC CTT GGA TTT CAG CGG CAC AAG GCT CAG CTG AAC CTG GCT        648
Met Ala Cys Leu Gly Phe Gln Arg His Lys Ala Gln Leu Asn Leu Ala
  1               5                  10                  15

GCC AGG ACC TGG CCC TGC ACT CTC CTG TTT TTT CTT CTC TTC ATC CCT        696
Ala Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
             20                  25                  30

GTC TTC TGC AAA GCA ATG CAC GTG GCC CAG CCT GCT GTG GTA CTG GCC        744
Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
         35                  40                  45

AGC AGC CGA GGC ATC GCC AGC TTT GTG TGT GAG TAT GCA TCT CCA GGC        792
Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
     50                  55                  60

AAA GCC ACT GAG GTC CGG GTG ACA GTG CTT CGG CAG GCT GAC AGC CAG        840
Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
 65                  70                  75                  80

GTG ACT GAA GTC TGT GCG GCA ACC TAC ATG ACG GGG AAT GAG TTG ACC        888
Val Thr Glu Val Cys Ala Ala Thr Tyr Met Thr Gly Asn Glu Leu Thr
                 85                  90                  95

TTC CTA GAT GAT TCC ATC TGC ACG GGC ACC TCC AGT GGA AAT CAA GTG        936
Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
            100                 105                 110

AAC CTC ACT ATC CAA GGA CTG AGG GCC ATG GAC ACG GGA CTC TAC ATC        984
Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
        115                 120                 125

TGC AAG GTG GAG CTC ATG TAC CCA CCG CCA TAC TAC CTG GGC ATA GGC       1032
Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly
    130                 135                 140

AAC GGA ACC CAG ATT TAT GTA ATT GAT CCA GAA CCG TGC CCA GAT TCT       1080
Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser
145                 150                 155                 160
```

GAC GCT GAG CCC AAA TCG GCC GAC 1104
Asp Ala Glu Pro Lys Ser Ala Asp
165

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 168 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ala Cys Leu Gly Phe Gln Arg His Lys Ala Gln Leu Asn Leu Ala
1 5 10 15

Ala Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
20 25 30

Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
35 40 45

Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
50 55 60

Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
65 70 75 80

Val Thr Glu Val Cys Ala Ala Thr Tyr Met Thr Gly Asn Glu Leu Thr
85 90 95

Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
100 105 110

Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
115 120 125

Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly
130 135 140

Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser
145 150 155 160

Asp Ala Glu Pro Lys Ser Ala Asp
165

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro Thr His Val Asn Val Ser Val Val Met Ala Glu Val Asp Gly Thr
1 5 10 15

Cys Tyr

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ser Leu Ser Pro Gly Lys
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Pro Thr Leu Tyr Asn Val Ser Leu Val Met Ser Asp Thr Ala Gly Thr
1 5 10 15

Cys Tyr

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Pro Thr His Val Asn Val Ser Val Val Met Ala Glu Val Asp Gly Thr
1 5 10 15

Cys Tyr

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gly Pro Ser Lys Pro Glu Pro Lys Ser Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Asn Lys Ile Leu
1

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

His Phe Pro Asp Gln Glu Pro Lys Ser Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Val Ile His Val
1

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Pro Pro Pro Asp His Glu Pro Lys Ser Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Leu Lys Ile Gln
1

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Glu Pro Cys Pro Asp Ser Asp Ala Glu Pro Lys Ser Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met His Val Ala
1

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CCCAAATCGG CCGACAAAAC T 21

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TCAGCGAGCT CTAGACTACA CTCATTTACC CGGAGACAAG CTTAGGCTCT TCTGCGT 57

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 79 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AGCTTGTCTG CGGGTAAACC CACCCATGTC AATGTGTCTG TTGTCATGGC GGAGGTGGAC 60

GGCACCTGCT ACTGATAGT 79

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 79 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTAGACTATC AGTAGCAGGT GCCGTCCACC TCCGCCATGA CAACAGACAC ATTGACATGG 60

GTGGGTTTAC CCGCAGACA 79

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gly Pro Ser Lys Pro Glu Pro Lys Ser Ala Gly Ile Lys Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ser Leu Ser Thr Gly Lys
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ser Leu Ser Ala Gly Lys
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Glu Pro Lys Ser Ala
1 5

What is claimed is:

1. A hexameric fusion protein comprising:

(a) a dimeric binding protein and

(b) a tailpiece αtp) characterized by having the activity of the tailpiece from the C-terminus of the heavy chain of an IgA antibody.

2. The fusion protein according to claim 1, wherein the dimeric binding protein is selected from the group consisting of:

(a) a protein fragment comprising the extracellular domain of a selected monomeric binding protein or a functional fragment thereof fused to an Ig-Fc fragment selected from the group consisting of an Fc fragment from an IgG antibody, an Fc fragment from an IgD antibody, an Fc fragment from an IgE antibody, and an Fc fragment from an IgM antibody excluding the μtp; and

(b) a naturally dimeric binding protein or a fragment thereof having the binding ability of said dimeric protein.

3. The fusion protein according to claim 2 further comprising a leader suitable for expression and processing of the fusion protein.

4. The fusion protein according to claim 2 wherein the protein fragment consists of the native leader and extracellular domains selected from the group consisting of CD80, CTLA-4 and CD86.

5. The fusion protein according to claim 2 wherein the dimeric binding protein is a Ig-Fab fragment and the heavy chain is joined to the Ig-Fc fragment.

6. The fusion protein according to claim 2 wherein the Ig-Fc fragment is from an IgG antibody selected from the group of human isotypes consisting of IgG_1, IgG_2, IgG_3, IgG_4, and IgG binding mutants.

7. The fusion protein according to claim 2 wherein the Ig-Fc fragment is from a human IgG_1 antibody.

8. The fusion protein according to claim 1 wherein the αtp is the tailpiece f an antibody selected from the group consisting of human IgA1, human IgA2, rabbit IgA, mouse IgA, and gorilla IgG.

9. The fusion protein according to claim 8 wherein the αtp has the sequence SEQ ID NO: 10 PTHVNVSVVMAEVDGTCY.

10. The fusion protein according to claim 8 wherein the αtp has been modified to remove the N-linked glycosylation site.

11. The fusion protein according to claim 1 further comprising a linker of between 1 to about 20 amino acids in length, said linker located between the binding protein and the αtp.

12. The fusion protein according to claim 1 which is a homo-hexamer.

13. The fusion protein according to claim 1 which is a hetero-hexamer.

14. A polynucleotide sequence encoding a hexameric fusion protein comprising:

(a) a dimeric binding protein and

(b) a tailpiece (αtp) characterized by having the biological activity of the tailpiece from the C-terminus of the heavy chain of an IgA antibody.

15. The polynucleotide sequence according to claim 14, wherein the dimeric binding protein is selected from the group consisting of:

(a) a protein fragment comprising the extracellular domain of a selected monomeric binding protein fused to an Ig-Fc fragment selected from the group consisting of an Fc fragment from an IgG antibody, an Fc fragment from an IgD antibody, an Fc fragment from an IgE antibody, and an Fc fragment from an IgM antibody excluding the μtp; and

(b) a naturally dimeric binding protein or a fragment thereof having the binding ability of said protein.

16. The polynucleotide sequence according to claim 15 further comprising a leader suitable for expression and processing of the fusion protein.

17. The polynucleotide sequence according to claim 15 wherein the protein fragment consists of the native leader and extracellular domains selected from the group consisting of CD80, CTLA-4 and CD86.

18. The polynucleotide sequence according to claim 15 wherein the dimeric protein is a Ig-Fab fragment and the heavy chain is joined to the Ig-Fc fragment.

19. The polynucleotide sequence according to claim 15 wherein the Ig-Fc fragment is from an IgG antibody selected from the group of human isotypes consisting of IgG_1, IgG_2, IgG_3, IgG_4, and IgG binding mutants.

20. The polynucleotide sequence according to claim 15 wherein the Ig-Fc fragment is from a human IgG_1 antibody.

21. The polynucleotide sequence according to claim 14 wherein the αtp is the tailpiece of an antibody selected from the group consisting of human IgA1, human IgA2, rabbit IgA, mouse IgA, and gorilla IgG.

22. The polynucleotide sequence according to claim 21 wherein the αtp has the sequence SEQ ID NO: 101 THVNVSVVMAEVDGTCY.

23. The polynucleotide sequence according to claim 21 wherein the αtp has been modified to remove the N-linked glycosylation site.

24. The polynucleotide sequence according to claim 14, wherein the fusion protein further comprises a linker of between 1 to about 20 amino acids in length, said linker located between the binding protein and the αtp.

25. A vector comprising a polynucleotide sequence encoding:

(a) a polynucleotide sequence according to claim 14; and

(b) sequences controlling expression of the fusion protein in a selected host cell.

26. A recombinant host cell comprising the vector of claim 25.

27. A pharmaceutical composition comprising an hexameric fusion protein according to claim 1 in a pharmaceutically acceptable carrier.

28. A pharmaceutical composition comprising a polynucleotide sequence according to claim 14 in a pharmaceutically acceptable carrier.

29. A diagnostic reagent comprising a detectable label and an hexameric fusion protein according to claim 1.

30. A diagnostic reagent comprising a detectable label and a polynucleotide sequence according to claim 14.

31. A method for producing a hexameric fusion protein comprising the steps of:

(a) providing a dimeric binding protein; and

(b) attaching to each monomer of said binding protein a tailpiece (αtp) characterized by having the biological activity of the tailpiece from the C-terminus of the heavy chain of an IgA antibody.

32. A method of purifying a hexameric fusion protein comprising:

(a) providing a selected host cell according to claim 26;

(b) recovering the stable hexameric fusion protein; and

(c) purifying the recovered fusion protein.

33. The method according to claim 32, wherein said fusion protein comprises IgG or a fragment thereof, and said purification step comprises the step of applying said fusion protein to a Protein A or Protein G column.

34. A method for screening for a ligand which binds to a hexameric fusion protein according to claim 1, comprising the steps of:

(a) providing the hexameric fusion protein;

(b) permitting a test sample to come into contact with the hexameric fusion protein; and

(c) detecting binding between the fusion protein and any ligand in the test sample.

35. The method according to claim 34 wherein the fusion protein is immobilized to a surface.

36. The method according to claim 34 wherein the fusion protein is in solution.

37. The method according to claim 34, wherein the fusion protein is selected from the group consisting of CD80-Igαtp and CD86-Igαtp.

38. A method for screening for a compound that inhibits the interaction between a selected binding protein and a ligand, said method comprising the step of

(a) providing a known ligand for said binding protein;

(b) providing a hexameric fusion protein according to claim 1;

(c) contacting the known ligand with a test solution;

(d) contacting the known ligand with the hexameric fusion protein;

(e) detecting inhibition of binding of the hexameric fusion protein; and

(f) optionally isolating the compound which binds to the hexameric protein.

39. The method according to claim 38, wherein the ligand is selected from the group consisting of CD28 and CTLA-4 and the hexameric fusion protein is selected from the group consisting of CD80-Igαtp and CD86-Igαtp.

40. A method for stimulating CD28 positive cells comprising the step of administering to CD28 positive cells a hexameric fusion protein selected from the group consisting of CD80-Igαtp and CD86-Igαtp.

41. A method for suppressing CTLA-4 positive cells comprising the step of administering to CTLA-4 positive cells a hexameric fusion protein selected from the group consisting of CD80-Igαtp and CD86-Igαtp.

42. A method for antagonizing cell surface CD80- and CD86-mediated stimulation of CD28 positive cells by administering to said cells a hexameric fusion protein CTLA4-Igαtp.

43. A method for immunizing an animal comprising the method of administering to the animal an effective amount of a pharmaceutical compositions according to claim 27.

44. A method for immunizing an animal comprising the method of administering to the animal an effective amount of a pharmaceutical compositions according to claim 28.

FIG. 1

Hexameric CD80-Igαtp

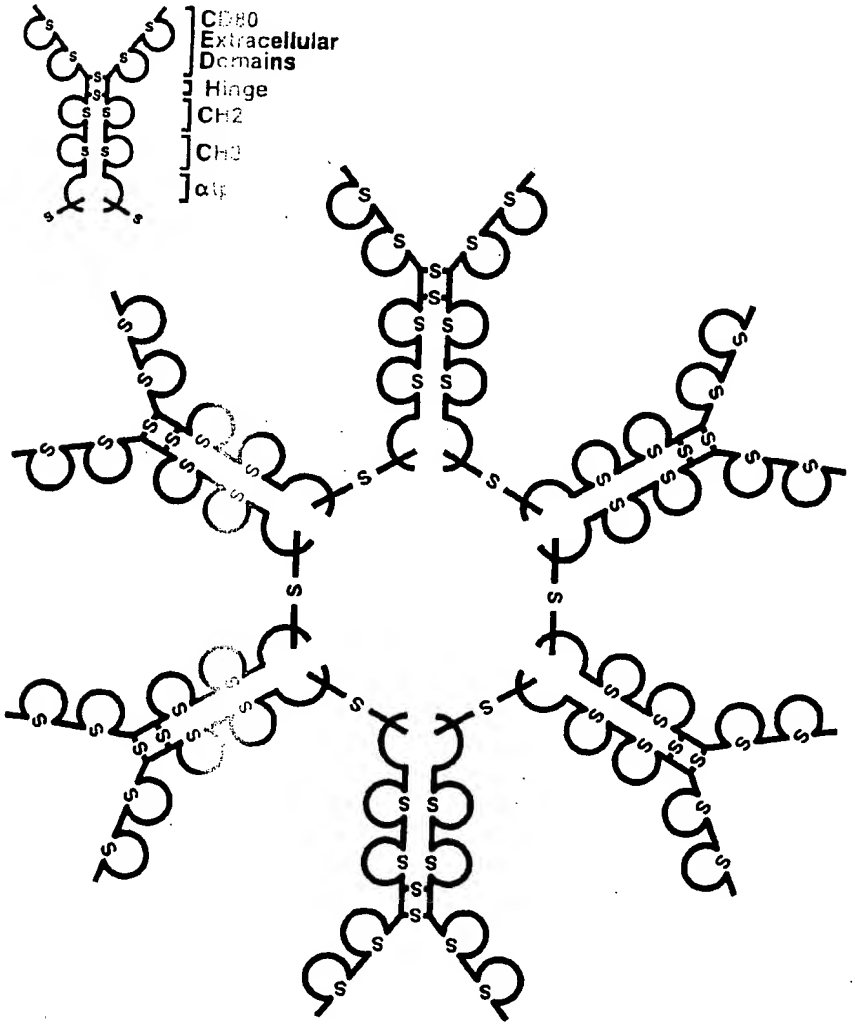


FIG. 2

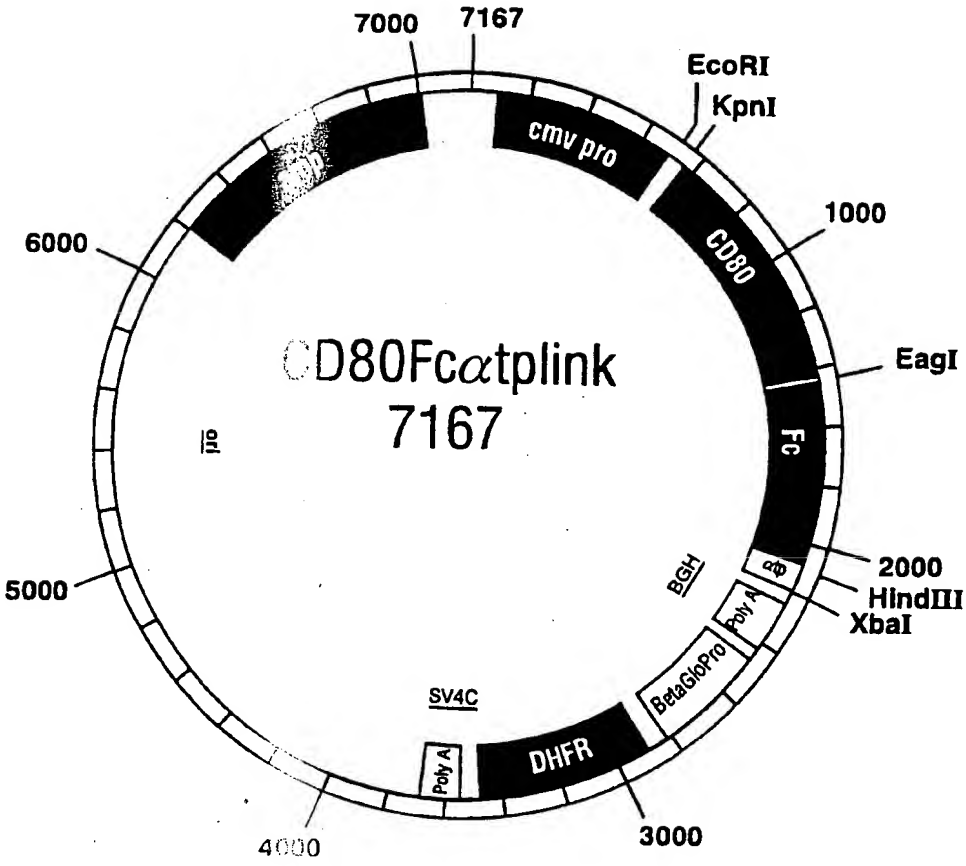

7000
7167
EcoRI
KpnI
cmv pro
CD80
1000
6000
CD80Fcαtplink
7167
EagI
Fc
ori
2000
HindIII
XbaI
5000
BGH
Poly A
BetaGloPro
SV4C
DHFR
Poly A
4000
3000

FIG. 3

DNA sequence of CD8-Fcαtplink

```
   1  GACGTCGAC  GATCGGGAGA TCGGGGATCG ATCCGTCGAC GTACGACTAG
  51  TTATTAATA  TAATCAATTA CGGGGTCATT AGTTCATAGC CCATATATGG
 101  AGTTCCGCC  TACATAACTT ACGGTAAATG GCCCGCCTGG CTGACCGCCC
 151  AACGACCCC  GCCCATTGAC GTCAATAATG ACGTATGTTC CCATAGTAAC
 201  GCCAATAGG  ACTTTCCATT GACGTCAATG GGTGGACTAT TTACGGTAAA
 251  CTGCCCACT  GGCAGTACAT CAAGTGTATC ATATGCCAAG TACGCCCCCT
 301  ATTGACGTC  ATGACGGTAA ATGGCCCGCC TGGCATTATG CCCAGTACAT
 351  GACCTTATG  GACTTTCCTA CTTGGCAGTA CATCTACGTA TTAGTCATCG
 401  CTATTACCA  GGTGATGCGG TTTTGGCAGT ACATCAATGG GCGTGGATAG
 451  CGGTTTGAC  CACGGGGATT TCCAAGTCTC CACCCCATTG ACGTCAATGG
 501  GAGTTTGTT  TGGCACCAAA ATCAACGGGA CTTTCCAAAA TGTCGTAACA
 551  ACTCCGCCC  ATTGACGCAA ATGGGCGGTA GGCGTGTACG GTGGGAGGTC
 601  TATATAAGC  GAGCTGGGTA CGTGAACCGT CAGATCGCCT GGAGACGCCA
 651  TCGAATTCG  TTACCTGCAG ATATCAAGCT AATTCGGTAC CGGGCCCCCC
 701  TCGAGCCTC  AGCCATGGGC CACACACGGA GGCAGGGAAC ATCACCATCC
 751  AAGTGTCCA  ACCTCAATTT CTTTCAGCTC TTGGTGCTGG CTGGTCTTTC
 801  TCACTTCTG  TCAGGTGTTA TCCACGTGAC CAAGGAAGTG AAAGAAGTGG
 851  CAACGCTGT  CTGTGGTCAC AATGTTTCTG TTGAAGAGCT GGCACAAACT
 901  CGCATCTAC  GGCAAAAGGA GAAGAAAATG GTGCTGACTA TGATGTCTGG
```

FIG. 3A

951 GGACATGAAT ATATGGCCCG AGTACAAGAA CCGGACCATC TTTGATATCA

1001 CTAATAACCT CTCCATTGTG ATCCTGGCTC TGCGCCCATC TGACGAGGGC

1051 ACATACGA GTGTTGTTCT GAAGTATGAA AAAGACGCTT TCAAGCGGGA

1101 ACACCTGG GAAGTGACGT TATCAGTCAA AGCTGACTTC CCTACACCTA

1151 GTATATCT CTTTGAAATT CCAACTTCTA ATATTAGAAG GATAATTTGC

1201 TCAACCTCT GAGGTTTTCC AGAGCCTCAC CTCTCCTGGT TGGAAAATGG

1251 AGAAGAAT AATGCCATCA ACACAACAGT TTCCCAAGAT CCTGAAACTG

1301 AGCTCTAT TGTTAGCAGC AAACTGGATT TCAATATGAC AACCAACCAC

1351 AGCTTCAT GTCTCATCAA GTATGGACAT TTAAGAGTGA ATCAGACCTT

1401 CAACTGGA ACAACCAAGC AAGAGCATTT TCCTGATCAG GAGCCCAAAT

1451 CGGCCGAC AACTCACACA TGCCCACCGT GCCCAGCACC TGAACTCCTG

1501 GGGGGACC CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ACACCCTCAT

1551 GATCTCCC ACCCCTGAGG TCACATGCGT GGTGGTGGAC GTGAGCCACG

1601 AAGACCCTG GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT

1651 AATGCCAAG CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGGGT

1701 GGTCAGCGT TCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT

1751 ACAAGTGCA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC

1801 ATCTCCAAA CAAAGGGCA GCCCCGAGAA CCACAGGTGT ACACCCTGCC

1851 CCCATCCCG ATGAGCTGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG

1901 TCAAAGGCT TATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG

FIG. 3B

1951 CAGCCGGA ACAACTACAA GACCACGCCT CCCGTGCTGG ACTCCGACGG

2001 CTCCTTCT CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC

2051 AGGGGAAC CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC

2101 TACACGCA AGAGCCTAAG CTTGTCTGCG GGTAAACCCA CCCATGTCAA

2151 TGTGTCTG GTCATGGCGG AGGTGGACGG CACCTGCTAC TGATAGTCTA

2201 GAGCTCGC ATCAGCCTCG ACTGTGCCTT CTAGTTGCCA GCCATCTGTT

2251 GTTTGCCC CCCCCGTGCC TTCCTTGACC CTGGAAGGTG CCACTCCCAC

2301 TGTCCTTT TAATAAAATG AGGAAATTGC ATCGCATTGT CTGAGTAGGT

2351 GTCATTCT TCTGGGGGGT GGGGTGGGGC AGGACAGCAA GGGGGAGGAT

2401 TGGGAAGA ATAGCAGGCA TGCTGGGGAT GCGGTGGGCT CTATGGAACC

2451 AGCTGGGG CGAGGGGGGA TCTCCCGATC CCCAGCTTTG CTTCTCAATT

2501 TCTTATTT ATAATGAGAA AAAAAGGAAA ATTAATTTTA ACACCAATTC

2551 AGTAGTTG TGAGCAAATG CGTTGCCAAA AAGGATGCTT TAGAGACAGT

2601 GTTCTCTG CAGATAAGGA CAAACATTAT TCAGAGGGAG TACCCAGAGC

2651 TGAGACTC AAGCCAGTGA GTGGCACAGC ATTCTAGGGA GAAATATGCT

2701 TGTCATCA GAAGCCTGAT TCCGTAGAGC CACACCTTGG TAAGGGCCAA

2751 TCTGCTCA CAGGATAGAG AGGGCAGGAG CCAGGGCAGA GCATATAAGG

2801 TGAGGTAG TCAGTTGCTC CTCACATTTG CTTCTGACAT AGTTGTGTTG

2851 GGAGCTTG TAGCTTGGAC AGCTCAGGGC TGCGATTTCG CGCCAAACTT

2901 GACGGCAA CTAGCGTGAA GGCTGGTAGG ATTTTATCCC CGCTGCCATC

FIG. 3C

2951 ATGGTTCGAC CATTGAACTG CATCGTCGCC GTGTCCCAAA ATATGGGGAT

3001 TGGCAAGA GGAGACCTAC CCTGGCCTCC GCTCAGGAAC GAGTTCAAGT

3051 ACTTCCAA AATGACCACA ACCTCTTCAG TGGAAGGTAA ACAGAATCTG

3101 GTGATTAT GTAGGAAAAC CTGGTTCTCC ATTCCTGAGA AGAATCGACC

3151 TTTAAAGG AGAATTAATA TAGTTCTCAG TAGAGAACTC AAAGAACCAC

3201 CACGAGGA TCATTTTCTT GCCAAAAGTT TGGATGATGC CTTAAGACTT

3251 ATTGAACA CGGAATTGGC AAGTAAAGTA GACATGGTTT GGATAGTCGG

3301 AGGCAGTT GTTTACCAGG AAGCCATGAA TCAACCAGGC CACCTTAGAC

3351 TCTTTGTG AAGGATCATG CAGGAATTTG AAAGTGACAC GTTTTTCCCA

3401 GAAATTGA TGGGGAAATA TAAACTTCTC CCAGAATACC CAGGCGTCCT

3451 CTCTGAGG CAGGAGGAAA AAGGCATCAA GTATAAGTTT GAAGTCTACG

3501 AGAAGAAA CTAACAGGAA GATGCTTTCA AGTTCTCTGC TCCCCTCCTA

3551 AAGCTATG TTTTTATAAG ACCATGCTAG CTTGAACTTG TTTATTGCAG

3601 CTTATAATG TACAAATAA AGCAATAGCA TCACAAATTT CACAAATAAA

3651 GCATTTTTT CACTGCATTC TAGTTGTGGT TTGTCCAAAC TCATCAATGT

3701 ATCTTATCA GTCTGGATCA ACGATAGCTT ATCTGTGGGC GATGCCAAGC

3751 ACCTGGATG TGTTGGTTTC CTGCTACTGA TTTAGAAGCC ATTTGCCCCC

3801 TGAGTGGGG TTGGGAGCAC TAACTTTCTC TTTCAAAGGA AGCAATGCAG

3851 AAAGAAAAG ATACAAAGTA TAAGCTGCCA TGTAATAATG GAAGAAGATA

3901 AGGTTGTAT AATTAGATTT ACATACTTCT GAATTGAAAC TAAACACCTT

FIG. 3D

3951 TAAATTCT . AATATATAAC ACATTTCATA TGAAAGTATT TTACATAAGT

4001 AACTCAGA . CATAGAAAAC AAAGCTAATG ATAGGTGTCC CTAAAAGTTC

4051 ATTTATTA T TCTACAAATG ATGAGCTGGC CATCAAAATT CCAGCTCAAT

4101 TCTTCAAC ATTAGAAAGA GCAATCTGCA AACTCATCTG GAATAACAAA

4151 AAACCTAG TAGCAAAAAC TCTTCTCAAG GATAAAAGAA CCTCTGGTGG

4201 AATCACCA CCTGACCTAA AGCTGTACTA CAGAGCAATT GTGATAAAAA

4251 CTGCATGG CTGATATAGA AACGGACAAG TAGACCAATG GAATAGAACC

4301 CACACACC TGGTCACTTG ATCTTCAACA AGAGAGCTAA AACCATCCAC

4351 TGGAAAAA ACAGCATTTT CAACAAATGG TGCTGGCACA ACTGGTGGTT

4401 ATCATGGA AGAATGTGAA TTGATCCATT CCAATCTCCT TGTACTAAGG

4451 TCAAATCT TTGGATCAAG GAACTCCACA TAAAACCAGA GACACTGAAA

4501 CTTATAGA AGAAAGTGGG GAAAAGCCTC GAAGATATGG GCACAGGGGA

4551 AAAATTCC AATAGAACAG CAATGGCTTG TGCTGTAAGA TCGAGAATTG

4601 ACAAATGG CTCATGAAA CTCCAAAGCT ATCGGATCAA TTCCTCCAAA

4651 AAAGCCTC CACTACTTCT GGAATAGCTC AGAGGCCGAG GCGGCCTCGG

4701 CCTCTGCA AATAAAAAAA ATTAGTCAGC CATGCATGGG GCGGAGAATG

4751 GGCGGAAC GCGGAGTTA GGGGCGGGAT GGGCGGAGTT AGGGGCGGGA

4801 CTATGGTT GACTAATTG AGATGCATGC TTTGCATACT TCTGCCTGCT

4851 GGGGAGCC GGACTTTCC ACACCTGGTT GCTGACTAAT TGAGATGCAT

4901 GCTTTGCA TTCTGCCTG CTGGGGAGCC TGGGGACTTT CCACACCCTA

FIG. 3E

4951 ACTGACAC ATTCCACAGA ATTAATTCCC GATCCCGTCG ACCTCGAGAG

5001 CTTGGCGT CATGGTCAT AGCTGTTTCC TGTGTGAAAT TGTTATCCGC

5051 TCACAATT CACACAACATA CGAGCCGGAA GCATAAAGTG TAAAGCCTGG

5101 GGTGCCTA GAGTGAGCTA ACTCACATTA ATTGCGTTGC GCTCACTGCC

5151 CGCTTTCC TCGGGAAACC TGTCGTGCCA GCTGCATTAA TGAATCGGCC

5201 AACGCGCG AGAGGCGGT TTGCGTATTG GGCGCTCTTC CGCTTCCTCG

5251 CTCACTGA GCTGCGCTC GGTCGTTCGG CTGCGGCGAG CGGTATCAGC

5301 TCACTCAA CGGTAATAC GGTTATCCAC AGAATCAGGG GATAACGCAG

5351 GAAAGAAC TGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG

5401 GCCGCGTT GGCGTTTTT CCATAGGCTC CGCCCCCCTG ACGAGCATCA

5451 CAAAAATC GCTCAAGTC AGAGGTGGCG AAACCCGACA GGACTATAAA

5501 GATACCAG TTTCCCCCT GGAAGCTCCC TCGTGCGCTC TCCTGTTCCG

5551 ACCCTGCC TACCGGATA CCTGTCCGCC TTTCTCCCTT CGGGAAGCGT

5601 GGCGCTTT AATGCTCAC GCTGTAGGTA TCTCAGTTCG GTGTAGGTCG

5651 TTCGCTCC TGGGCTGT GTGCACGAAC CCCCCGTTCA GCCCGACCGC

5701 TGCGCCTT CGGTAACTA TCGTCTTGAG TCCAACCCGG TAAGACACGA

5751 CTTATCGC TGGCAGCAG CCACTGGTAA CAGGATTAGC AGAGCGAGGT

5801 ATGTAGGC GCTACAGAG TTCTTGAAGT GGTGGCCTAA CTACGGCTAC

5851 ACTAGAAG AGTATTTGG TATCTGCGCT CTGCTGAAGC CAGTTACCTT

5901 CGGAAAAAG TTGGTAGCT CTTGATCCGG CAAACAAACC ACCGCTGGTA

FIG. 3F

5951 GCGGTGGT TTTTGTTTGC AAGCAGCAGA TTACGCGCAG AAAAAAAGGA

6001 TCTCAAGA ATCCTTTGAT CTTTTCTACG GGGTCTGACG CTCAGTGGAA

6051 CGAAAACT CGTTAAGGGA TTTTGGTCAT GAGATTATCA AAAAGGATCT

6101 TCACCTAG CCTTTTAAAT TAAAAATGAA GTTTTAAATC AATCTAAAGT

6151 ATATATGA AAACTTGGTC TGACAGTTAC CAATGCTTAA TCAGTGAGGC

6201 ACCTATCT GCGATCTGTC TATTTCGTTC ATCCATAGTT GCCTGACTCC

6251 CCGTCGTG ATAACTACG ATACGGGAGG GCTTACCATC TGGCCCCAGT

6301 GCTGCAAT TACCGCGAGA CCCACGCTCA CCGGCTCCAG ATTTATCAGC

6351 AATAAACC CAGCCGGAA GGGCCGAGCG CAGAAGTGGT CCTGCAACTT

6401 TATCCGCC ATCCAGTCT ATTAATTGTT GCCGGGAAGC TAGAGTAAGT

6451 AGTTCGCC TAATAGTTT GCGCAACGTT GTTGCCATTG CTACAGGCAT

6501 CGTGGTGT GCTCGTCGT TTGGTATGGC TTCATTCAGC TCCGGTTCCC

6551 AACGATCA CGAGTTACA TGATCCCCCA TGTTGTGCAA AAAAGCGGTT

6601 AGCTCCTT TCCTCCGAT CGTTGTCAGA AGTAAGTTGG CCGCAGTGTT

6651 ATCACTCAT TTATGGCAG CACTGCATAA TTCTCTTACT GTCATGCCAT

6701 CCGTAAGAT TTTTCTGTG ACTGGTGAGT ACTCAACCAA GTCATTCTGA

6751 GAATAGTGT GCGGCGACC GAGTTGCTCT TGCCCGGCGT CAATACGGGA

6801 TAATACCGC ACATAGCA GAACTTTAAA AGTGCTCATC ATTGGAAAAC

6851 GTTCTTCGGG GAAAACTC TCAAGGATCT TACCGCTGTT GAGATCCAGT

6901 TCGATGTAAC ACTCGTGC ACCCAACTGA TCTTCAGCAT CTTTTACTTT

FIG. 3G

6951 CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT GCCGCAAAAA

7001 AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT CTTCCTTTTT

7051 CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATACAT

7101 ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTC

7151 CCCGAAAAGT GCCACCT

FIG. 4

Sequence and translation of the CD80αtp coding region in the CD80Fcαtp expression vector

```
          Eco RI                                                    Kpn I
          GAATTCGGTTAC  GCAGATATCAAGCTAATTCGGTACCGGGCCCCCCTCGAGCCTGAAG
653 -------+----  ----+---------+---------+---------+---------+-- 712
          CTTAAGCCAATG  CGTCTATAGTTCGATTAAGCCATGGCCCGGGGGGAGCTCGGACTTC

          CCATGGGCCACA  CGGAGGCAGGGAACATCACCATCCAAGTGTCCATACCTCAATTTCT
713 -------+----  ----+---------+---------+---------+---------+-- 772
          GGTACCCGGTGT  GCCTCCGTCCCTTGTAGTGGTAGGTTCACAGGTATGGAGTTAAAGA
            Initiation codon
            M  G  H  T  R  R  Q  G  T  S  P  S  K  C  P  Y  L  N  F  F -

          TTCAGCTCTTGG  CTGGCTGGTCTTTCTCACTTCTGTTCAGGTGTTATCCACGTGACCA
773 -------+----  ----+---------+---------+---------+---------+-- 832
          AAGTCGAGAACC  GACCGACCAGAAAGAGTGAAGACAAGTCCACAATAGGTGCACTGGT
                                                    Mature Peptide Seq.
            Q  L  L  V  L  A  G  L  S  H  F  C  S  G  V  I  H  V  T  K -

          AGGAAGTGAAAG  GTGGCAACGCTGTCCTGTGGTCACAATGTTTCTGTTGAAGAGCTGG
833 -------+----  ----+---------+---------+---------+---------+-- 892
          TCCTTCACTTTC  CACCGTTGCGACAGGACACCAGTGTTACAAAGACAACTTCTCGACC
            E  V  K  E  V  A  T  L  S  C  G  H  N  V  S  V  E  E  L  A -

          CACAAACTCGCA  TACTGGCAAAAGGAGAAGAAAATGGTGCTGACTATGATGTCTGGGG
893 -------+----  ----+---------+---------+---------+---------+-- 952
          GTGTTTGAGCGT  ATGACCGTTTTCCTCTTCTTTTACCACGACTGATACTACAGACCCC
            Q  T  R  I  Y  W  Q  K  E  K  K  M  V  L  T  M  M  S  G  D -

          ACATGAATATAT  CCCGAGTACAAGAACCGGACCATCTTTGATATCACTAATAACCTCT
953 -------+----  ----+---------+---------+---------+---------+-- 1012
          TGTACTTATATA  GGGCTCATGTTCTTGGCCTGGTAGAAACTATAGTGATTATTGGAGA
            M  N  I  W  P  E  Y  K  N  R  T  I  F  D  I  T  N  N  L  S -

          CCATTGTGATCC  GCTCTGCGCCCATCTGACGAGGGCACATACGAGTGTGTTGTTCTGA
1013 -------+----  ----+---------+---------+---------+---------+-- 1072
          GGTAACACTAGG  CGAGACGCGGGTAGACTGCTCCCGTGTATGCTCACACAACAAGACT
            I  V  I  L  A  L  R  P  S  D  E  G  T  Y  E  C  V  V  L  K -
```

FIG. 4A

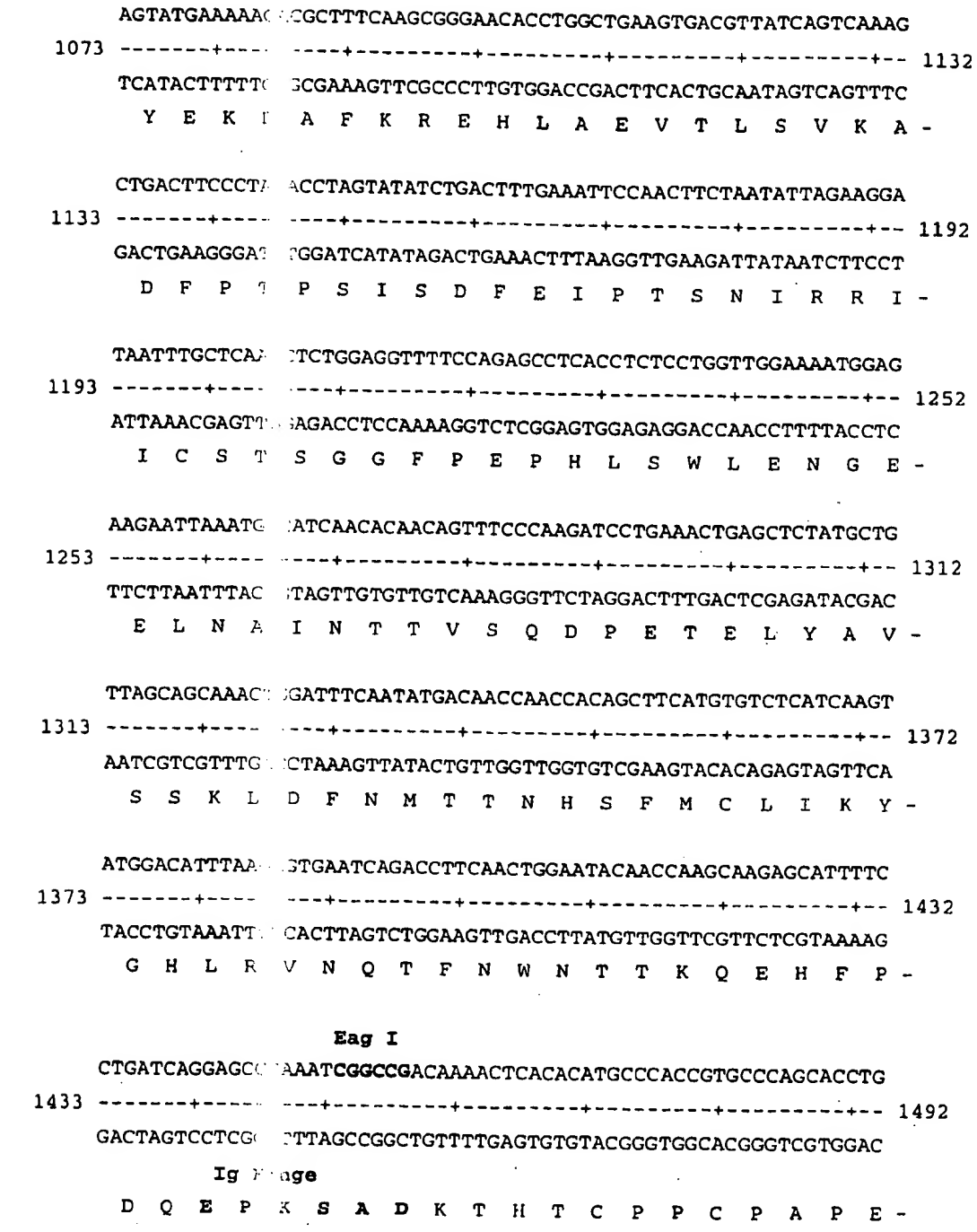


FIG. 4B

```
     AACTCCTGGGGG ACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGA
1493 -------+---- ---+---------+---------+---------+---------+-- 1552
     TTGAGGACCCCC TGGCAGTCAGAAGGAGAAGGGGGGTTTTGGGTTCCTGTGGGAGTACT
      L  L  G  G  P  S  V  F  L  F  P  P  K  P  K  D  T  L  M  I -

     TCTCCCGGACCC GAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGG
1553 -------+---- ---+---------+---------+---------+---------+-- 1612
     AGAGGGCCTGGG CTCCAGTGTACGCACCACCACCTGCACTCGGTGCTTCTGGGACTCC
      S  R  T  P  E  V  T  C  V  V  V  D  V  S  H  E  D  P  E  V -

     TCAAGTTCAACT TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG
1613 -------+---- ---+---------+---------+---------+---------+-- 1672
     AGTTCAAGTTGA ATGCACCTGCCGCACCTCCACGTATTACGGTTCTGTTTCGGCGCCC
      K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K  T  K  P  R  E -

     AGGAGCAGTACA AGCACGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT
1673 -------+---- ---+---------+---------+---------+---------+-- 1732
     TCCTCGTCATGT TCGTGCATGGCCCACCAGTCGCAGGAGTGGCAGGACGTGGTCCTGA
      E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L  H  Q  D  W -

     GGCTGAATGGCA GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
1733 -------+---- ---+---------+---------+---------+---------+-- 1792
     CCGACTTACCGT CTCATGTTCACGTTCCAGAGGTTGTTTCGGGAGGGTCGGGGGTAGC
      L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P  I  E -

     AGAAAACCATCTC AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC
1793 -------+---- ---+---------+---------+---------+---------+-- 1852
     TCTTTTGGTAGAG TTTCGGTTTCCCGTCGGGGCTCTTGGTGTCCACATGTGGGACGGGG
      K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P -

     CATCCCGGGATGA TGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCT
1853 -------+---- ---+---------+---------+---------+---------+-- 1912
     GTAGGGCCCTACT ACTGGTTCTTGGTCCAGTCGGACTGGACGGACCAGTTTCCGAAGA
      S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y -

     ATCCCAGCGACAT CCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGA
1913 -------+---- ---+---------+---------+---------+---------+-- 1972
     TAGGGTCGCTGTA GGCACCTCACCCTCTCGTTACCCGTCGGCCTCTTGTTGATGTTCT
      P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T -
```

FIG. 4C

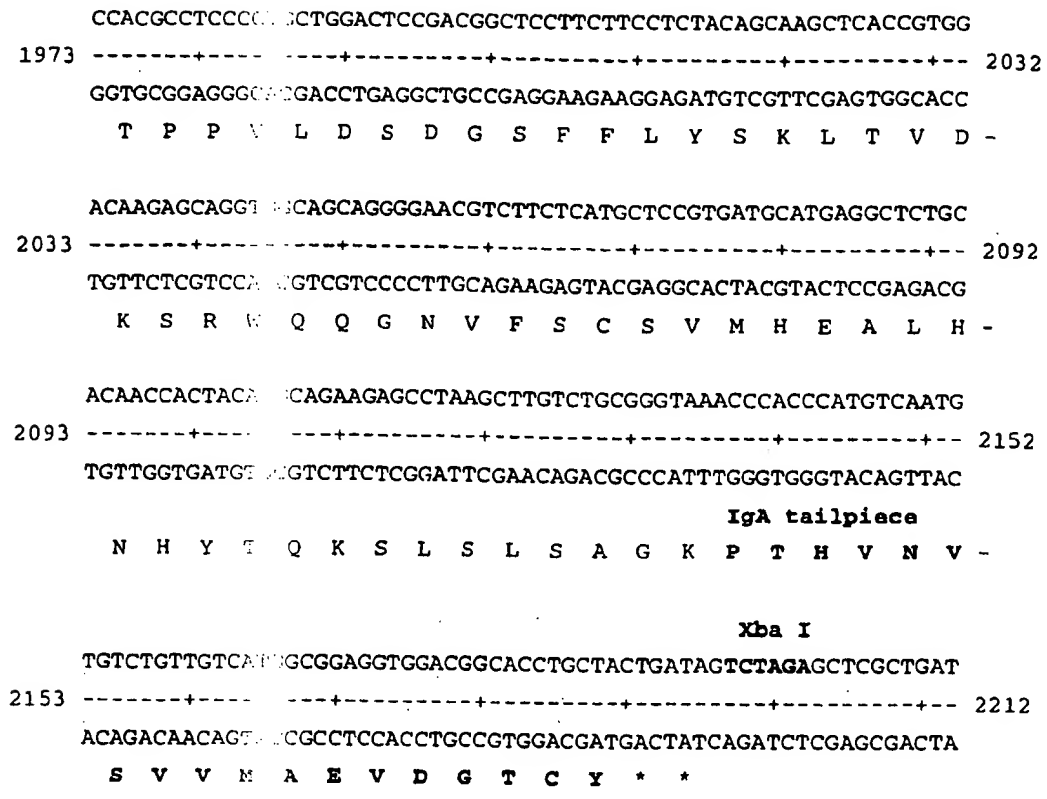

CCACGCCTCCCC CTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG
1973 2032
GGTGCGGAGGGC GACCTGAGGCTGCCGAGGAAGAAGGAGATGTCGTTCGAGTGGCACC
T P P L D S D G S F F L Y S K L T V D -
ACAAGAGCAGGT CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC
2033 2092
TGTTCTCGTCCA GTCGTCCCCTTGCAGAAGAGTACGAGGCACTACGTACTCCGAGACG
K S R W Q Q G N V F S C S V M H E A L H -
ACAACCACTACA CAGAAGAGCCTAAGCTTGTCTGCGGGTAAACCCACCCATGTCAATG
2093 2152
TGTTGGTGATGT GTCTTCTCGGATTCGAACAGACGCCCATTTGGGTGGGTACAGTTAC
IgA tailpiece
N H Y T Q K S L S L S A G K P T H V N V -
Xba I
TGTCTGTTGTCA GCGGAGGTGGACGGCACCTGCTACTGATAGTCTAGAGCTCGCTGAT
2153 2212
ACAGACAACAGT CGCCTCCACCTGCCGTGGACGATGACTATCAGATCTCGAGCGACTA
S V V M A E V D G T C Y * *

FIG. 5

Sequence and translation for region encoding the CD86 extracellular domain in the CD86IgαTp vector

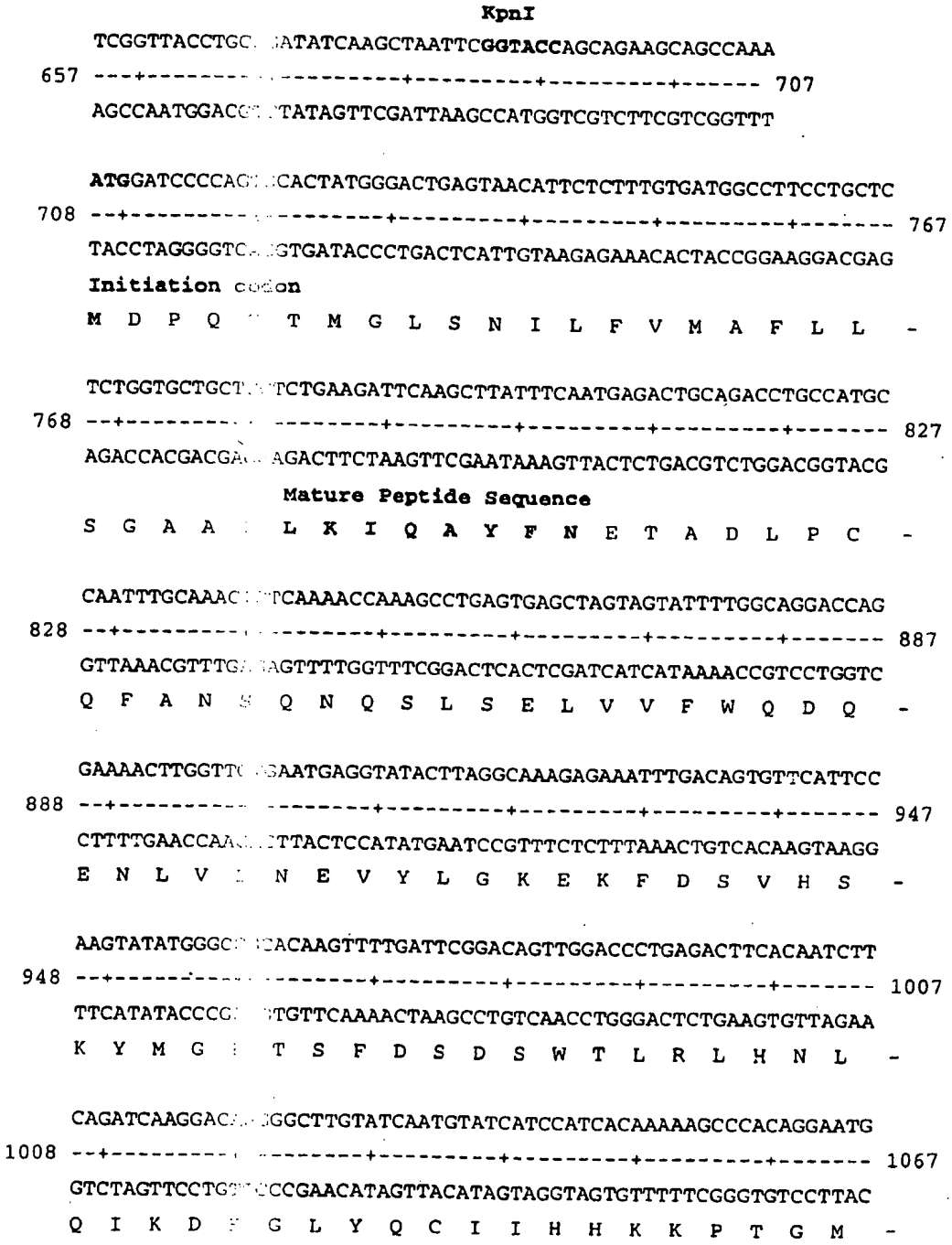


FIG. 5A

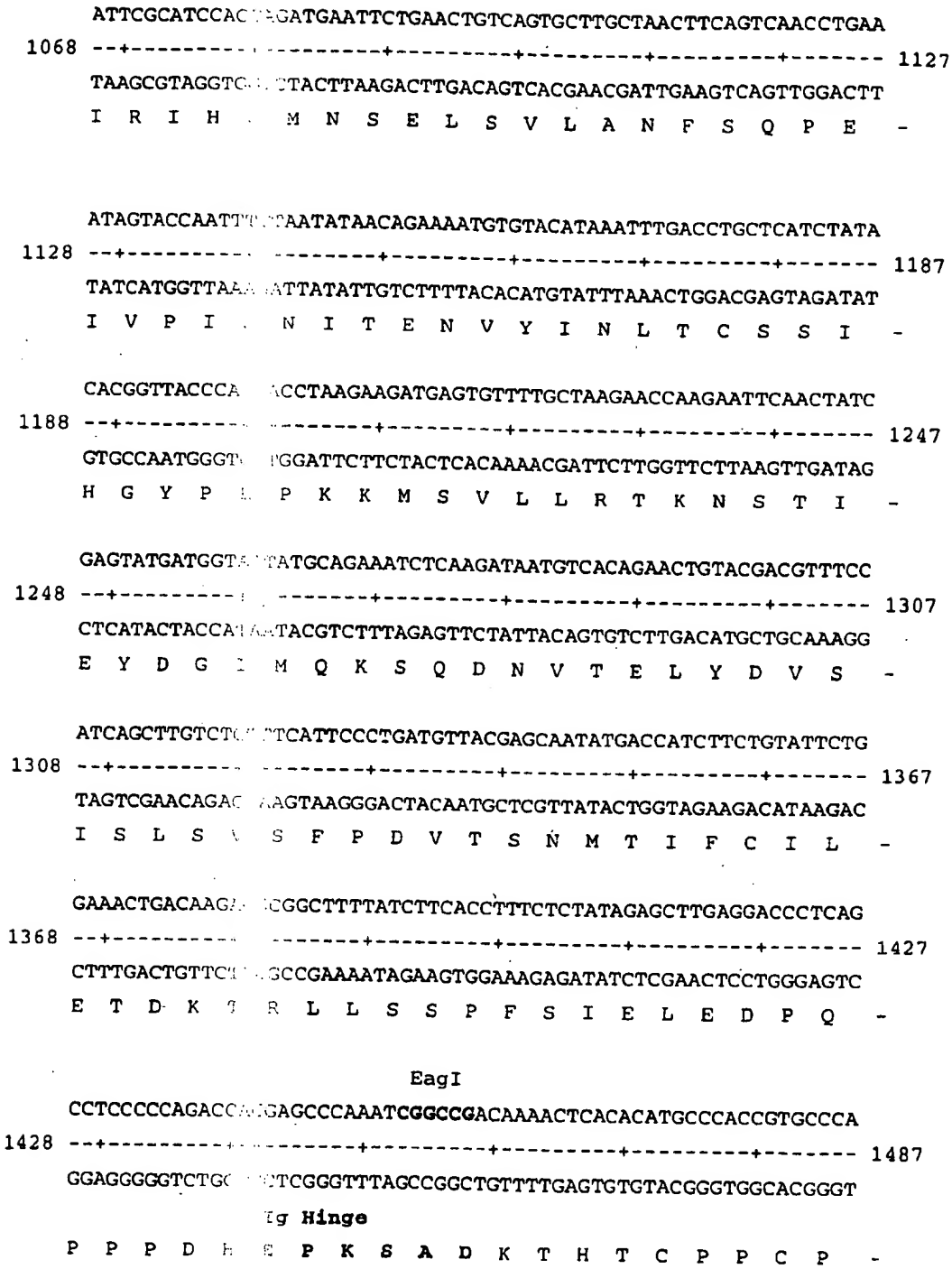

ATTCGCATCCAC GATGAATTCTGAACTGTCAGTGCTTGCTAACTTCAGTCAACCTGAA
1068 1127
TAAGCGTAGGTC CTACTTAAGACTTGACAGTCACGAACGATTGAAGTCAGTTGGACTT
I R I H M N S E L S V L A N F S Q P E -
ATAGTACCAATTT AATATAACAGAAAATGTGTACATAAATTTGACCTGCTCATCTATA
1128 1187
TATCATGGTTAA ATTATATTGTCTTTTACACATGTATTTAAACTGGACGAGTAGATAT
I V P I N I T E N V Y I N L T C S S I -
CACGGTTACCCA CCTAAGAAGATGAGTGTTTTGCTAAGAACCAAGAATTCAACTATC
1188 1247
GTGCCAATGGGT GGATTCTTCTACTCACAAAACGATTCTTGGTTCTTAAGTTGATAG
H G Y P P K K M S V L L R T K N S T I -
GAGTATGATGGT TATGCAGAAATCTCAAGATAATGTCACAGAACTGTACGACGTTTCC
1248 1307
CTCATACTACCA TACGTCTTTAGAGTTCTATTACAGTGTCTTGACATGCTGCAAAGG
E Y D G M Q K S Q D N V T E L Y D V S -
ATCAGCTTGTCT TCATTCCCTGATGTTACGAGCAATATGACCATCTTCTGTATTCTG
1308 1367
TAGTCGAACAGA AAGTAAGGGACTACAATGCTCGTTATACTGGTAGAAGACATAAGAC
I S L S S F P D V T S N M T I F C I L -
GAAACTGACAAG CGGCTTTTATCTTCACCTTTCTCTATAGAGCTTGAGGACCCTCAG
1368 1427
CTTTGACTGTTC GCCGAAAATAGAAGTGGAAAGAGATATCTCGAACTCCTGGGAGTC
E T D K R L L S S P F S I E L E D P Q -
EagI
CCTCCCCCAGACC GAGCCCAAATCGGCCGACAAAACTCACACATGCCCACCGTGCCCA
1428 1487
GGAGGGGGTCTGG TCGGGTTTAGCCGGCTGTTTTGAGTGTGTACGGGTGGCACGGGT
Ig Hinge
P P P D E P K S A D K T H T C P P C P -

FIG. 6

Sequence and translation for region encoding the CMV promoter and CTLA4 extracellular region in the CTLA4Igαtp vector.

```
                                                        SPEI
     GACGTCGACGG  GGAGATCGGGGATCGATCCGTCGACGTACGACTAGTTATTAATAG
   1 ---------+--  -----+---------+---------+---------+---------+ 60
     CTGCAGCTGCCT  CCTCTAGCCCCTAGCTAGGCAGCTGCATGCTGATCAATAATTATC

     TAATCAATTACG  GTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTT
  61 ---------+--  -----+---------+---------+---------+---------+
     ATTAGTTAATGC  CAGTAATCAAGTATCGGGTATATACCTCAAGGCGCAATGTATTGAA

     ACGGTAAATGGC  CCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATG
 121 ---------+--  -----+---------+---------+---------+---------+
     TGCCATTTACCG  GGACCGACTGGCGGGTTGCTGGGGGCGGGTAACTGCAGTTATTAC

     ACGTATGTTCCC  AGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTAT
 181 ---------+--  -----+---------+---------+---------+---------+
     TGCATACAAGGG  CATTGCGGTTATCCCTGAAAGGTAACTGCAGTTACCCACCTGATA

     TTACGGTAAACT  CACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT
 241 ---------+--  -----+---------+---------+---------+---------+
     AATGCCATTTGA  GGTGAACCGTCATGTAGTTCACATAGTATACGGTTCATGCGGGGGA

     ATTGACGTCAAT  GGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGG
 301 ---------+--  -----+---------+---------+---------+---------+
     TAACTGCAGTTA  CCATTTACCGGGCGGACCGTAATACGGGTCATGTACTGGAATACC

     GACTTTCCTACT  CAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGG
 361 ---------+--  -----+---------+---------+---------+---------+
     CTGAAAGGATGA  GTCATGTAGATGCATAATCAGTAGCGATAATGGTACCACTACGCC

     TTTTGGCAGTAC  AATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTC
 421 ---------+--  ----+---------+---------+---------+---------+
     AAAACCGTCATG  TACCCGCACCTATCGCCAAACTGAGTGCCCCTAAAGGTTCAGAG

     CACCCCATTGAC  AATGGGAGTTTGTTTTGGCGACTCACTATAGGAGTTCCCAAGCTT
 481 ---------+--  ----+---------+---------+---------+---------+
     GTGGGGTAACTG  TTACCCTCAAACAAAACCGCTGAGTGATATCCTCAAGGGTTCGAA
```

FIG. 6A

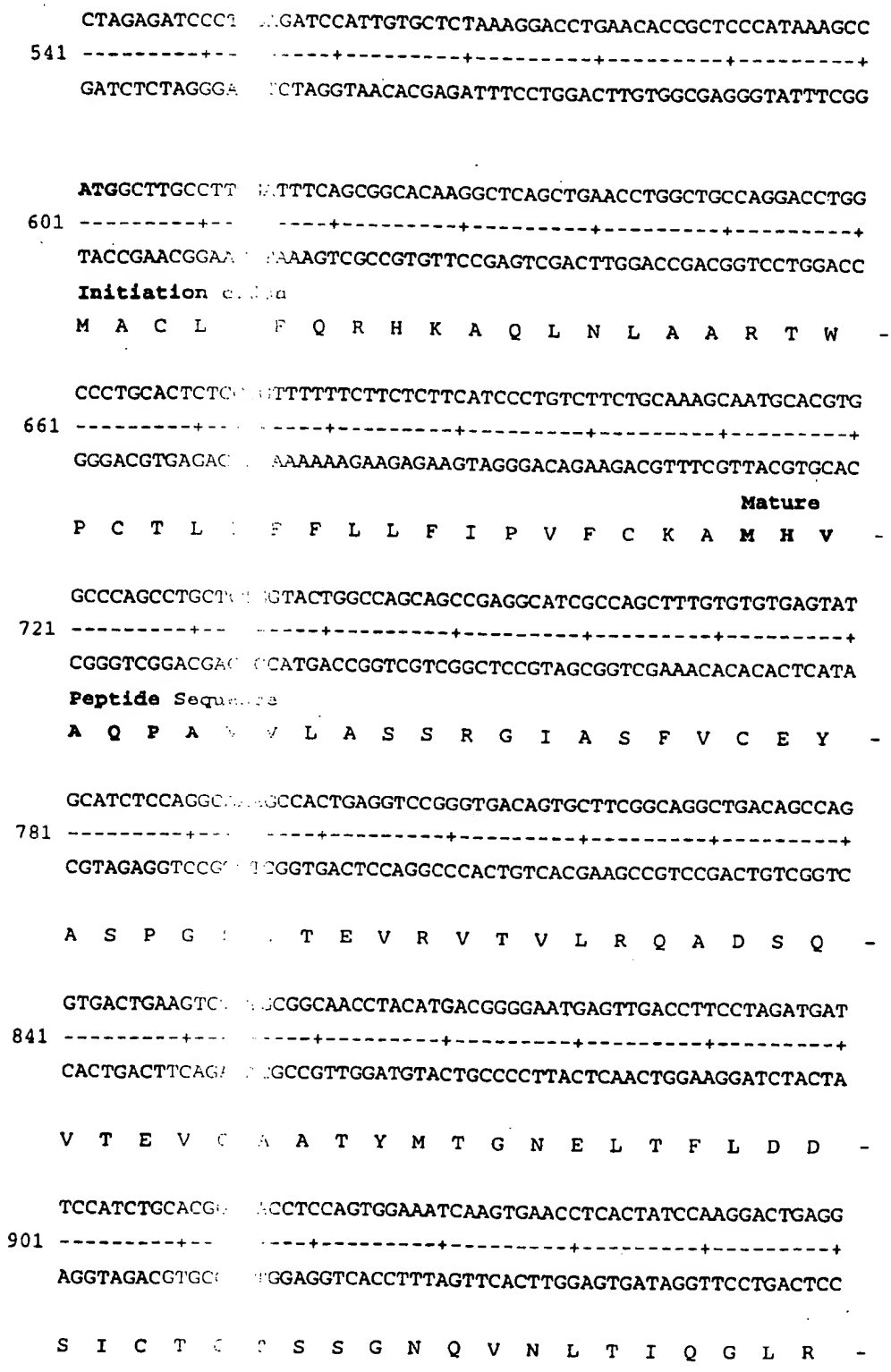

CTAGAGATCCCT GATCCATTGTGCTCTAAAGGACCTGAACACCGCTCCCATAAAGCC
541
GATCTCTAGGGA CTAGGTAACACGAGATTTCCTGGACTTGTGGCGAGGGTATTTCGG
ATGGCTTGCCTT TTTCAGCGGCACAAGGCTCAGCTGAACCTGGCTGCCAGGACCTGG
601
TACCGAACGGAA AAAGTCGCCGTGTTCCGAGTCGACTTGGACCGACGGTCCTGGACC
Initiation
M A C L F Q R H K A Q L N L A A R T W -
CCCTGCACTCTC TTTTTTTCTTCTCTTCATCCCTGTCTTCTGCAAAGCAATGCACGTG
661
GGGACGTGAGAC AAAAAAGAAGAGAAGTAGGGACAGAAGACGTTTCGTTACGTGCAC
Mature
P C T L F F L L F I P V F C K A M H V -
GCCCAGCCTGCT GTACTGGCCAGCAGCCGAGGCATCGCCAGCTTTGTGTGTGAGTAT
721
CGGGTCGGACGA CATGACCGGTCGTCGGCTCCGTAGCGGTCGAAACACACACTCATA
Peptide Sequence
A Q P A V L A S S R G I A S F V C E Y -
GCATCTCCAGGC GCCACTGAGGTCCGGGTGACAGTGCTTCGGCAGGCTGACAGCCAG
781
CGTAGAGGTCCG CGGTGACTCCAGGCCCACTGTCACGAAGCCGTCCGACTGTCGGTC
A S P G T E V R V T V L R Q A D S Q -
GTGACTGAAGTC CGGCAACCTACATGACGGGGAATGAGTTGACCTTCCTAGATGAT
841
CACTGACTTCAG GCCGTTGGATGTACTGCCCCTTACTCAACTGGAAGGATCTACTA
V T E V C A A T Y M T G N E L T F L D D -
TCCATCTGCACG CCTCCAGTGGAAATCAAGTGAACCTCACTATCCAAGGACTGAGG
901
AGGTAGACGTGC GGAGGTCACCTTTAGTTCACTTGGAGTGATAGGTTCCTGACTCC
S I C T S S S G N Q V N L T I Q G L R -

FIG. 6B

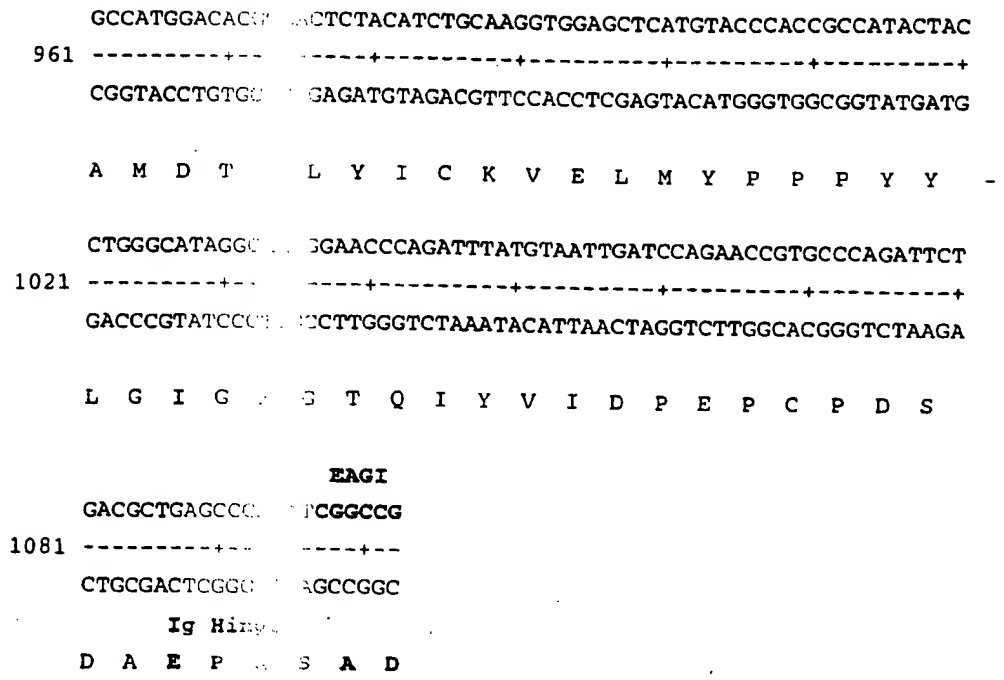

GCCATGGACAC CTCTACATCTGCAAGGTGGAGCTCATGTACCCACCGCCATACTAC
961 ----------+-- -----+---------+---------+---------+---------+
CGGTACCTGTG GAGATGTAGACGTTCCACCTCGAGTACATGGGTGGCGGTATGATG
A M D T L Y I C K V E L M Y P P P Y Y -
CTGGGCATAGG GAACCCAGATTTATGTAATTGATCCAGAACCGTGCCCAGATTCT
1021 ----------+-- ----+---------+---------+---------+---------+
GACCCGTATCC CCTTGGGTCTAAATACATTAACTAGGTCTTGGCACGGGTCTAAGA
L G I G G T Q I Y V I D P E P C P D S
EAGI
GACGCTGAGCC CGGCCG
1081 ----------+-- ----+--
CTGCGACTCGG GCCGGC
Ig Hin
D A E P S A D

FIG. 7

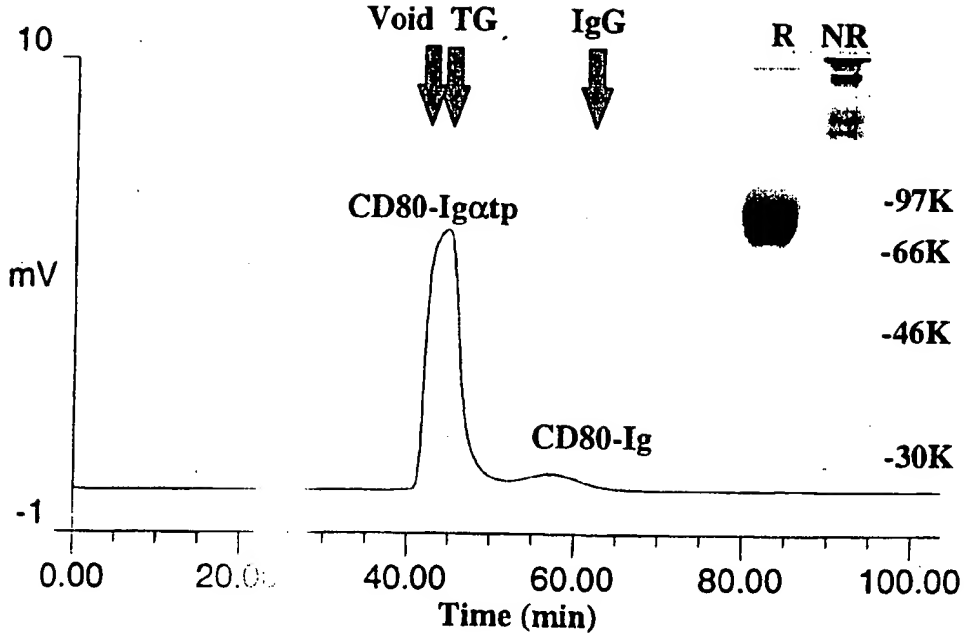

Void TG
IgG
R NR
10
mV
-1
CD80-Igαtp
CD80-Ig
-97K
-66K
-46K
-30K
0.00
20.00
40.00
60.00
80.00
100.00
Time (min)

FIG. 8

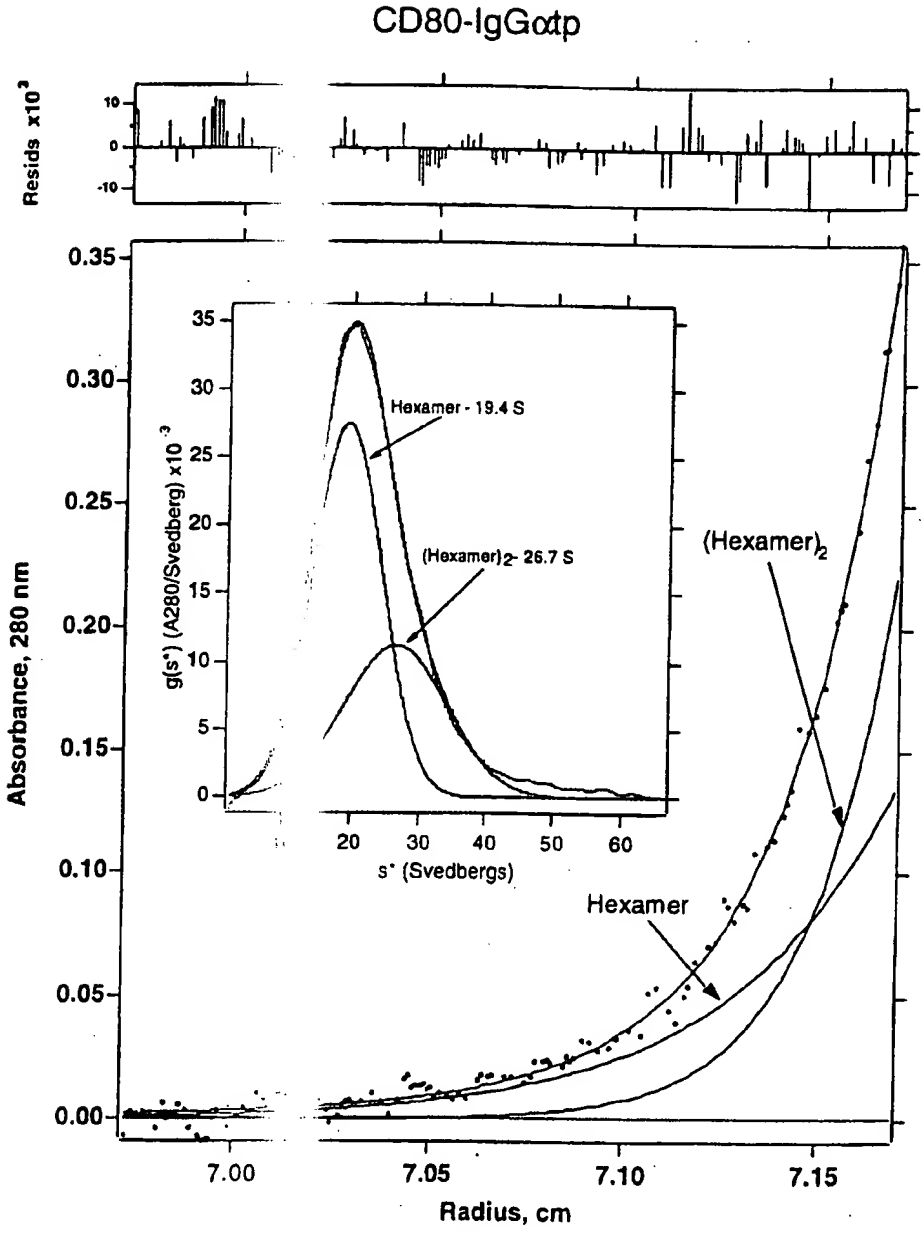

CD80-IgGαtp
Resids x10^3
10
0
-10
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Absorbance, 280 nm
7.00
7.05
7.10
7.15
Radius, cm
35
30
25
20
15
10
5
0
g(s*) (A280/Svedberg) x10^-3
Hexamer - 19.4 S
(Hexamer)2- 26.7 S
20
30
40
50
60
s* (Svedbergs)
(Hexamer)2
Hexamer

FIG. 9

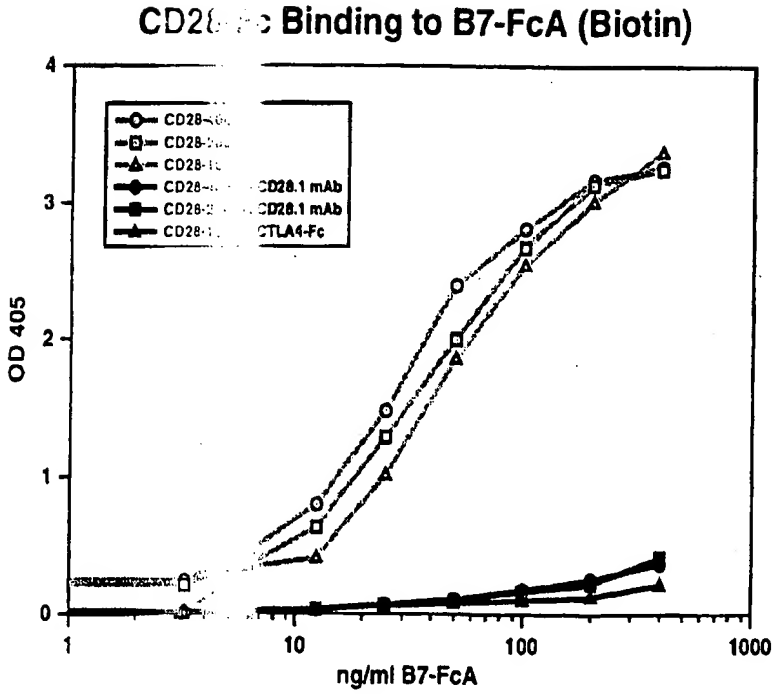

CD28 Fc Binding to B7-FcA (Biotin)
CD28-Fc
CD28-Fc
CD28-Fc
CD28-Fc CD28.1 mAb
CD28-Fc CD28.1 mAb
CD28-Fc CTLA4-Fc
OD 405
0
1
2
3
4
1
10
100
1000
ng/ml B7-FcA

FIG. 10

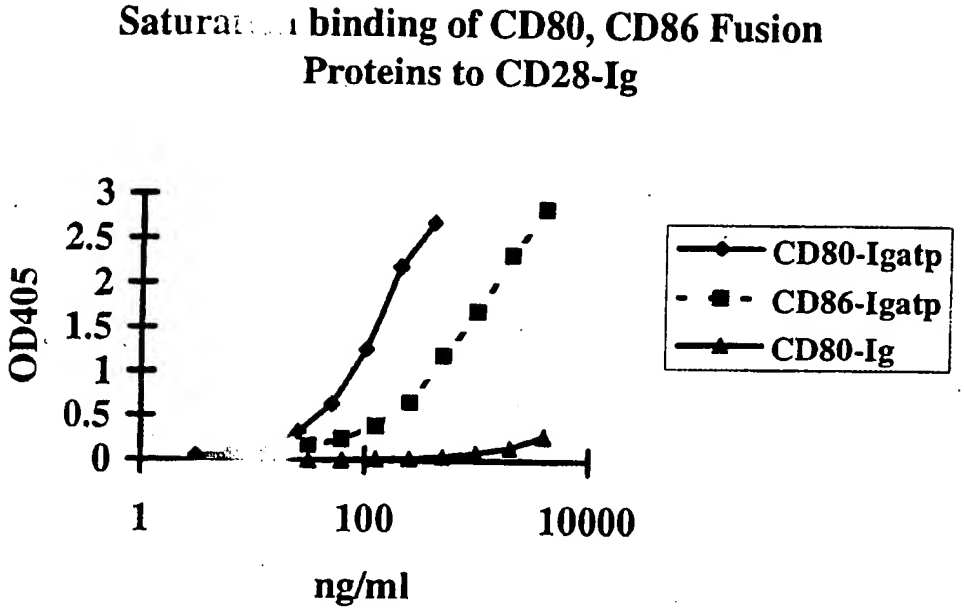

Saturation binding of CD80, CD86 Fusion Proteins to CD28-Ig
OD405
3
2.5
2
1.5
1
0.5
0
1
100
10000
ng/ml
CD80-Igatp
CD86-Igatp
CD80-Ig

FIG. 11

Saturation binding of CD80-Igαtp, CD86-Igαtp and CD80-Ig to CTLA4-Ig

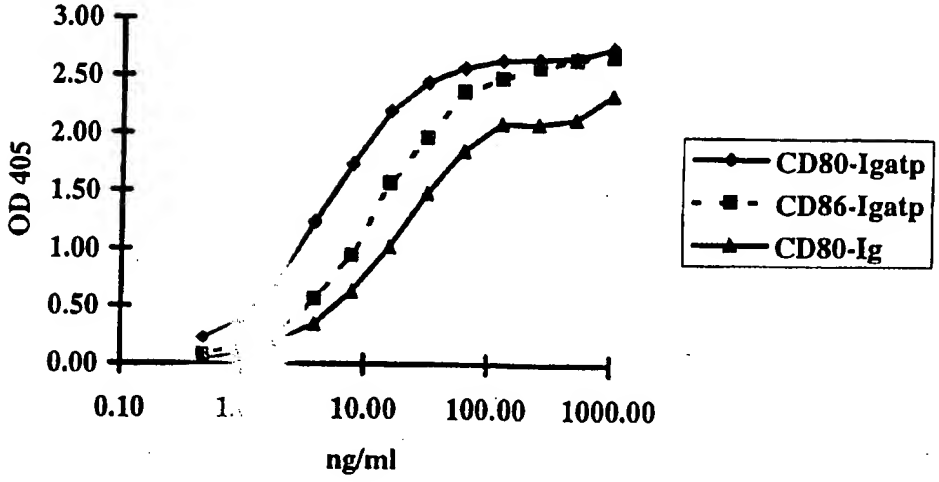


FIG. 12

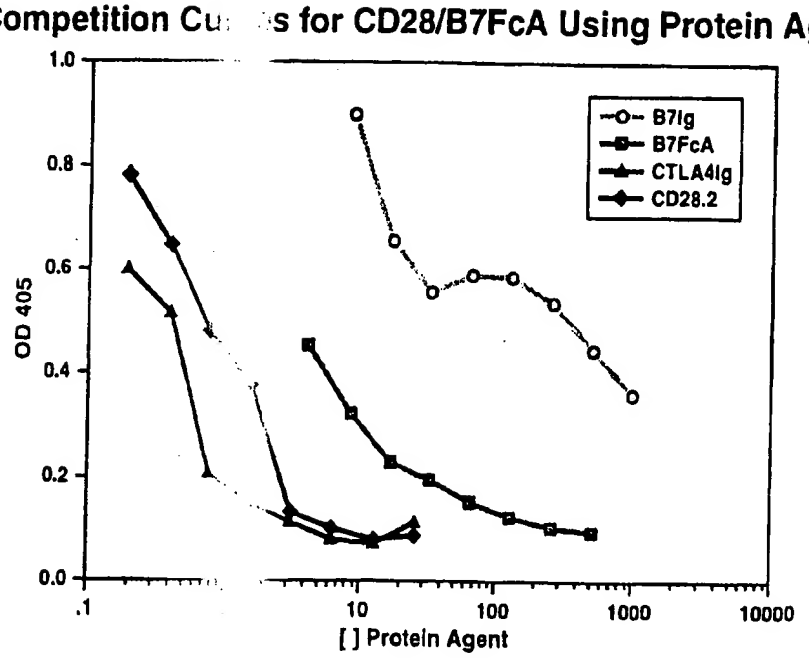
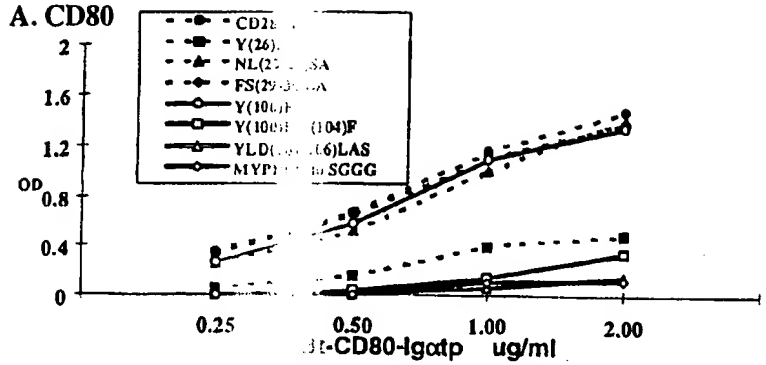
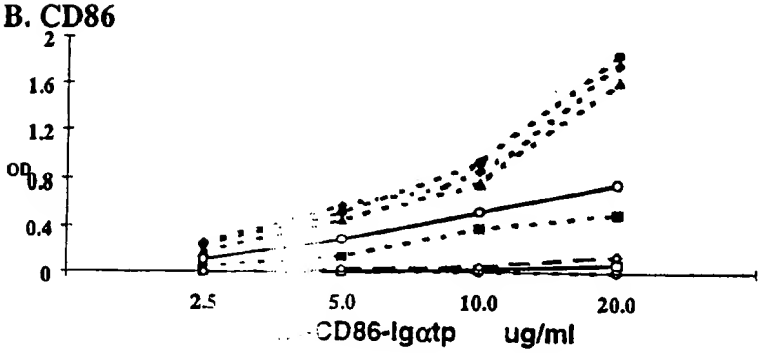

Competition Curves for CD28/B7FcA Using Protein Agents
B7Ig
B7FcA
CTLA4Ig
CD28.2
OD 405
1.0
0.8
0.6
0.4
0.2
0.0
.1
10
100
1000
10000
[ ] Protein Agent

FIG. 13

(A)


A. CD80
Y(26).
NL(2
FS(29
Y(100)F
Y(100
(104)F
YLD(
(6)LAS
MYP
SGGG
2
1.6
1.2
OD
0.8
0.4
0
0.25
0.50
1.00
2.00
-CD80-Igαtp ug/ml

(B)


B. CD86
2
1.6
1.2
OD
0.8
0.4
0
2.5
5.0
10.0
20.0
-CD86-Igαtp ug/ml

(C)

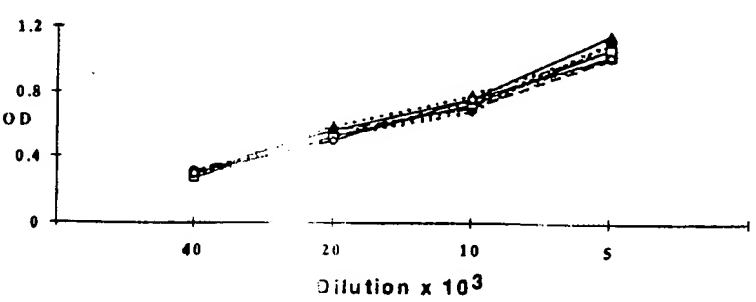

C. Rabbit Anti-CD2
era
1.2
0.8
OD
0.4
0
40
20
10
5
Dilution x 10³

FIG. 14

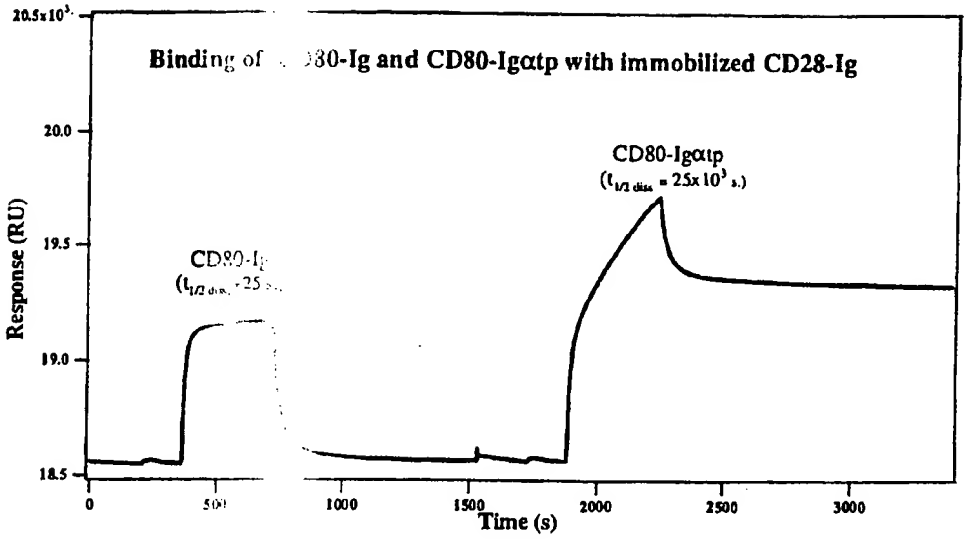

Binding of 80-Ig and CD80-Igαtp with immobilized CD28-Ig
CD80-Igαtp
Response (RU)
Time (s)
20.0
19.5
19.0
18.5
0
1000
1500
2000
2500
3000

FIG. 15

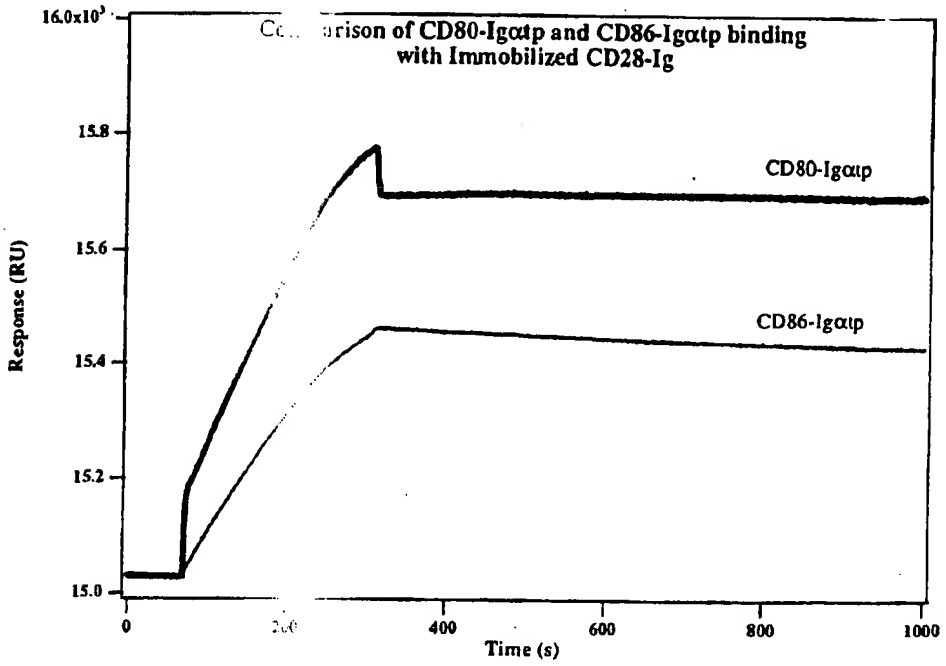
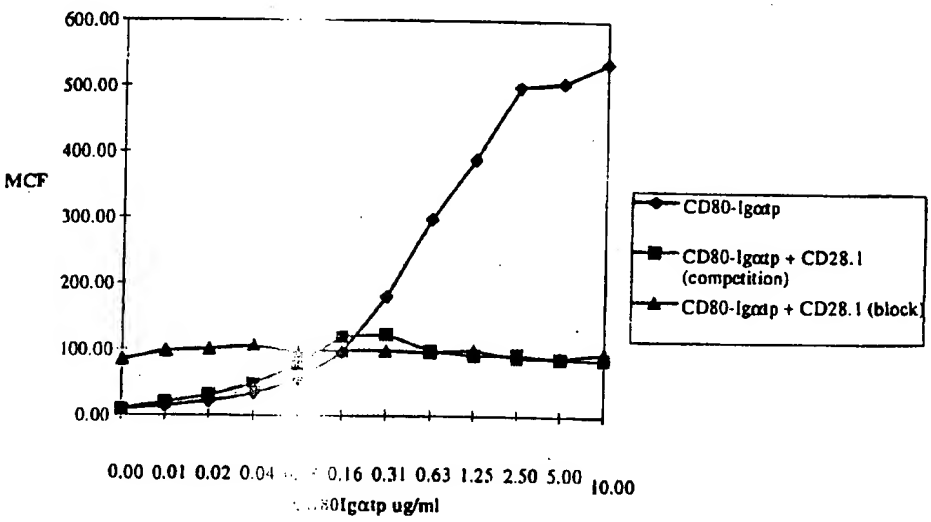

16.0x10³
15.8
15.6
15.4
15.2
15.0
Response (RU)
Comparison of CD80-Igαtp and CD86-Igαtp binding with Immobilized CD28-Ig
CD80-Igαtp
CD86-Igαtp
0
400
600
800
1000
Time (s)

FIG. 16

(A)

Flow cytometric analysis of CD80Igαtp binding to PCD28.1.S2.1 Cells



(B)

Flow cytometric analysis of CD86-Igαtp binding to PCD28.1.S2.1 Cells

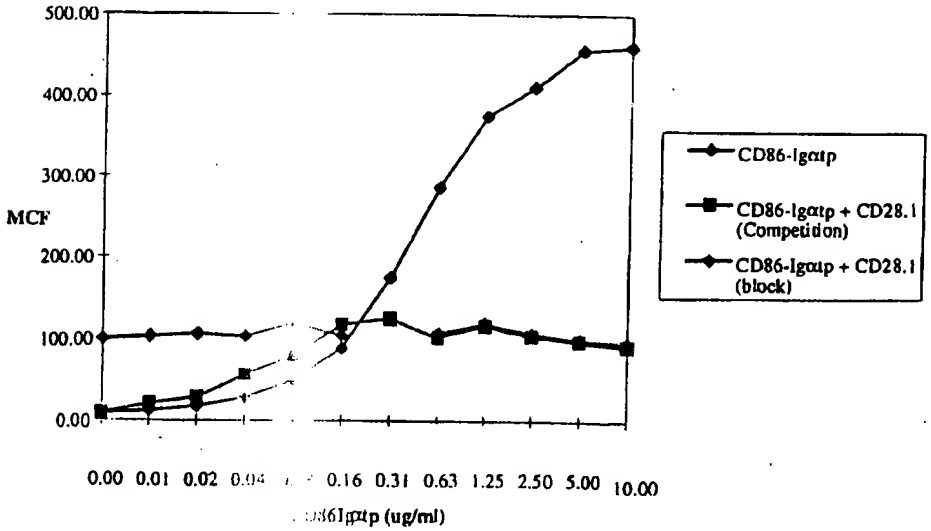


FIG. 17

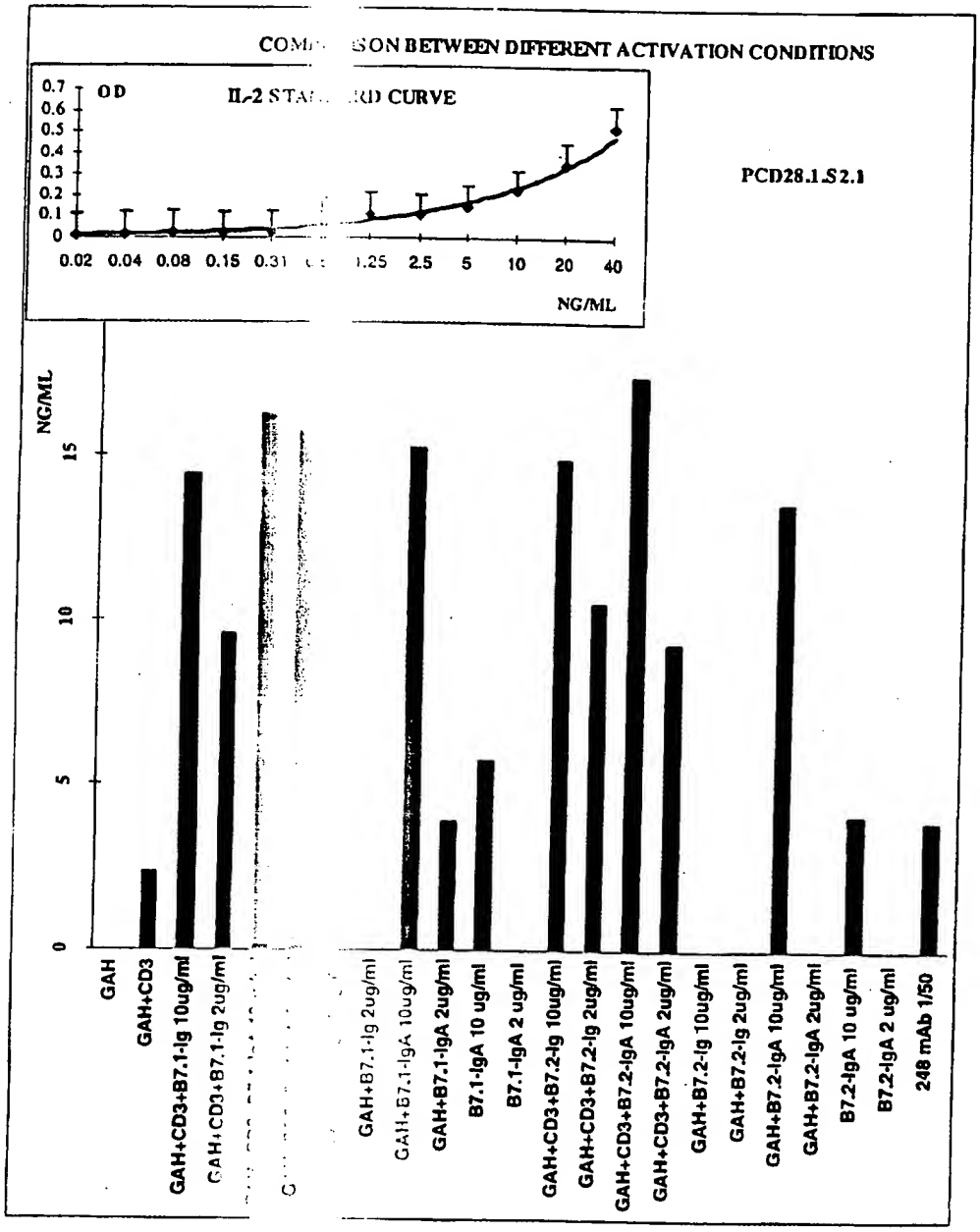

COMPARISON BETWEEN DIFFERENT ACTIVATION CONDITIONS
OD
IL-2 STANDARD CURVE
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.02 0.04 0.08 0.15 0.31
1.25 2.5 5 10 20 40
NG/ML
PCD28.1.S2.1
NG/ML
15
10
5
0
GAH
GAH+CD3
GAH+CD3+B7.1-Ig 10ug/ml
GAH+CD3+B7.1-Ig 2ug/ml
GAH+B7.1-Ig 2ug/ml
GAH+B7.1-IgA 10ug/ml
GAH+B7.1-IgA 2ug/ml
B7.1-IgA 10 ug/ml
B7.1-IgA 2 ug/ml
GAH+CD3+B7.2-Ig 10ug/ml
GAH+CD3+B7.2-Ig 2ug/ml
GAH+CD3+B7.2-IgA 10ug/ml
GAH+CD3+B7.2-IgA 2ug/ml
GAH+B7.2-Ig 10ug/ml
GAH+B7.2-Ig 2ug/ml
GAH+B7.2-IgA 10ug/ml
GAH+B7.2-IgA 2ug/ml
B7.2-IgA 10 ug/ml
B7.2-IgA 2 ug/ml
248 mAb 1/50

FIG. 18

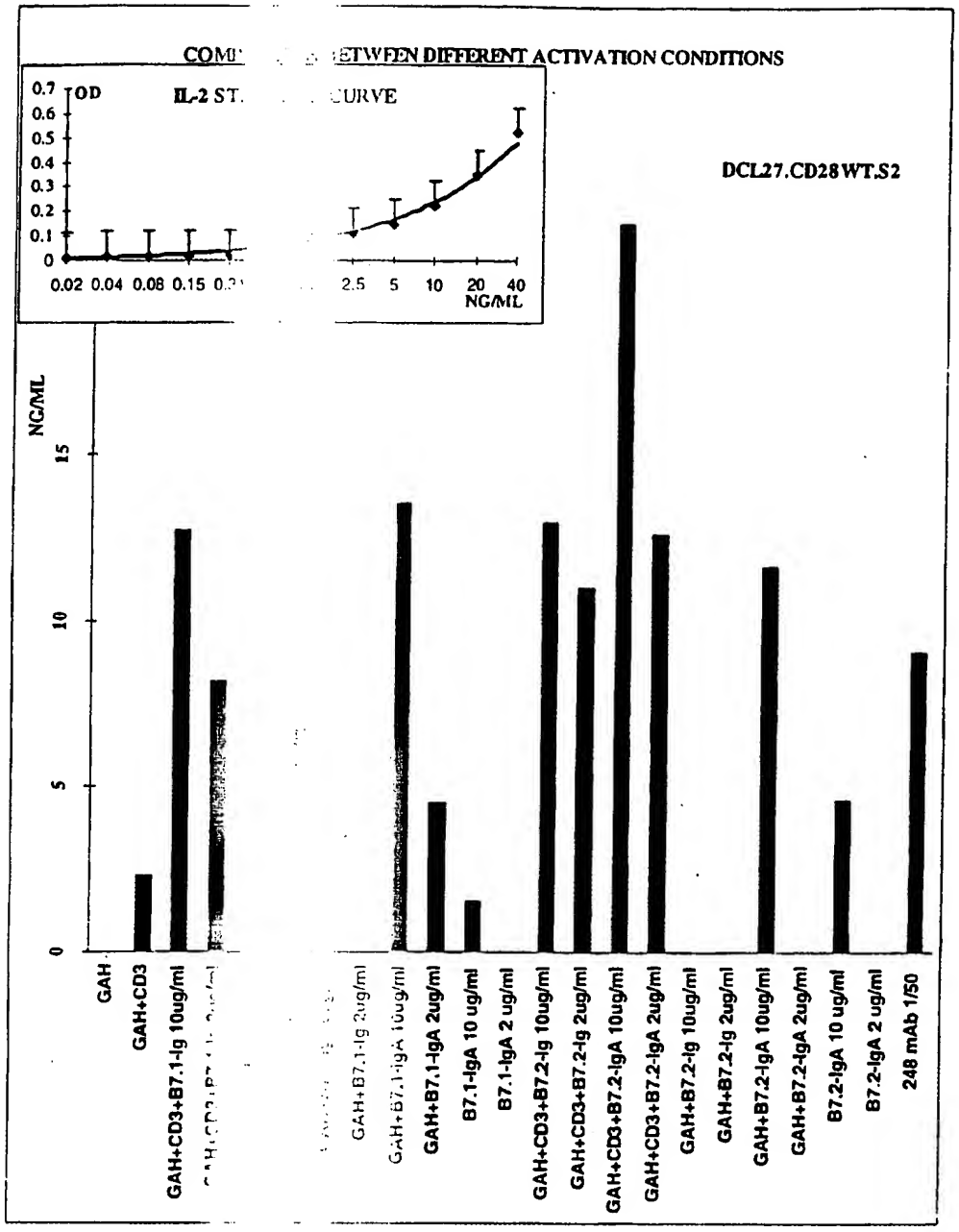

ETWEEN DIFFERENT ACTIVATION CONDITIONS
OD
IL-2 ST.
CURVE
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.02 0.04 0.08 0.15
2.5 5 10 20 40
NG/ML
DCL27.CD28WT.S2
NG/ML
15
10
5
0
GAH
GAH+CD3
GAH+CD3+B7.1-Ig 10ug/ml
GAH+B7.1-Ig 2ug/ml
GAH+B7.1-IgA 10ug/ml
GAH+B7.1-IgA 2ug/ml
B7.1-IgA 10 ug/ml
B7.1-IgA 2 ug/ml
GAH+CD3+B7.2-Ig 10ug/ml
GAH+CD3+B7.2-Ig 2ug/ml
GAH+CD3+B7.2-IgA 10ug/ml
GAH+CD3+B7.2-IgA 2ug/ml
GAH+B7.2-Ig 10ug/ml
GAH+B7.2-Ig 2ug/ml
GAH+B7.2-IgA 10ug/ml
GAH+B7.2-IgA 2ug/ml
B7.2-IgA 10 ug/ml
B7.2-IgA 2 ug/ml
248 mAb 1/50

FIG. 19

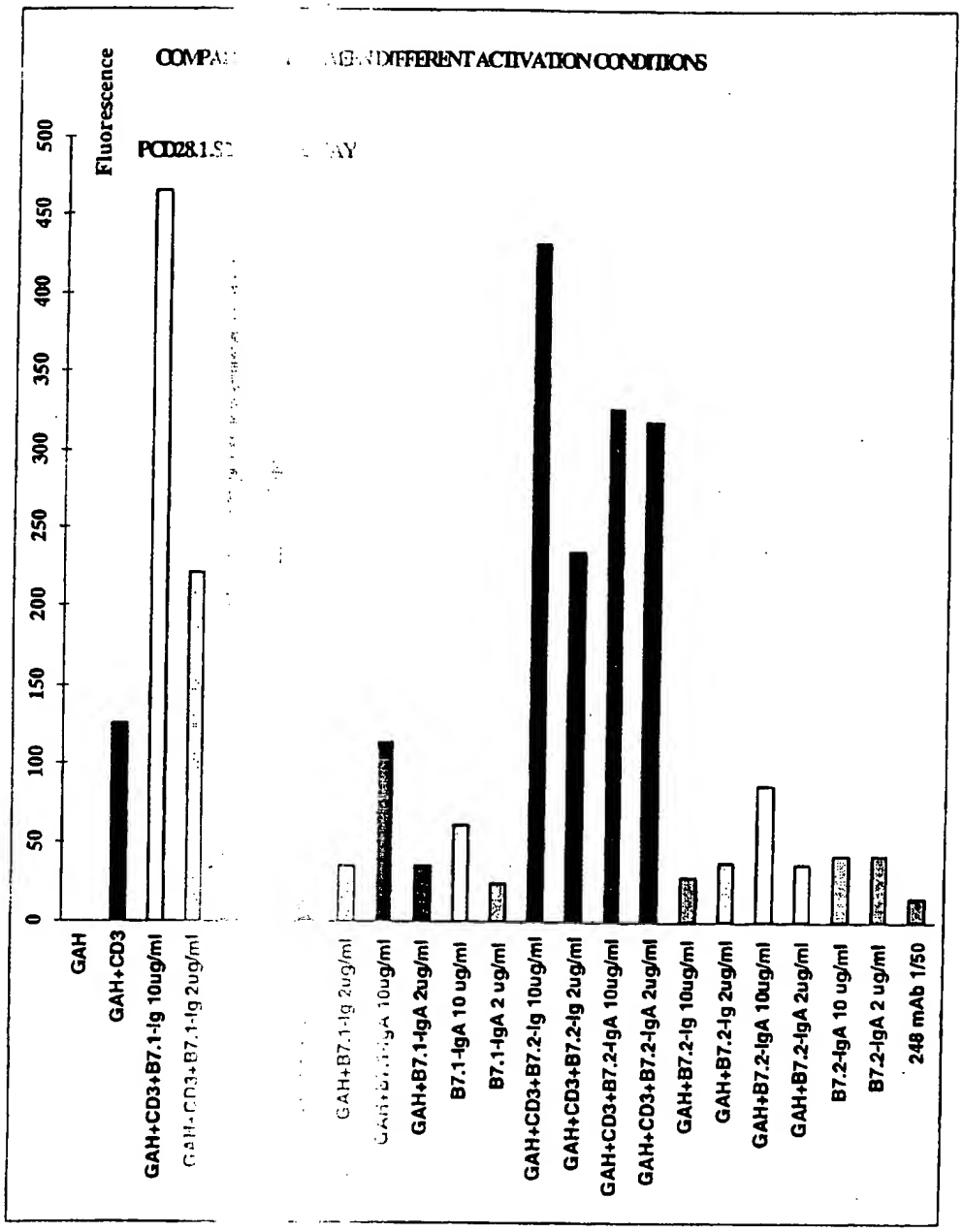
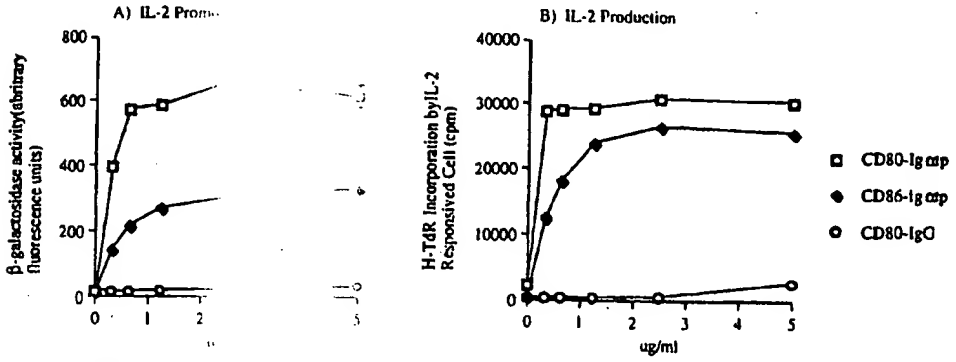
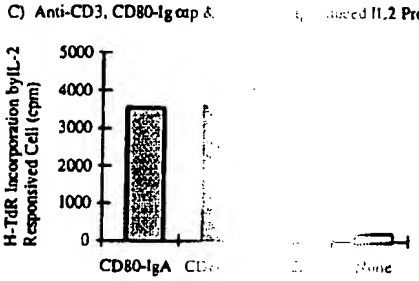

Fluorescence
0
50
100
150
200
250
300
350
400
450
500
GAH
GAH+CD3
GAH+CD3+B7.1-Ig 10ug/ml
GAH+CD3+B7.1-Ig 2ug/ml
GAH+B7.1-Ig 2ug/ml
GAH+B7.1-IgA 10ug/ml
GAH+B7.1-IgA 2ug/ml
B7.1-IgA 10 ug/ml
B7.1-IgA 2 ug/ml
GAH+CD3+B7.2-Ig 10ug/ml
GAH+CD3+B7.2-Ig 2ug/ml
GAH+CD3+B7.2-IgA 10ug/ml
GAH+CD3+B7.2-IgA 2ug/ml
GAH+B7.2-Ig 10ug/ml
GAH+B7.2-Ig 2ug/ml
GAH+B7.2-IgA 10ug/ml
GAH+B7.2-IgA 2ug/ml
B7.2-IgA 10 ug/ml
B7.2-IgA 2 ug/ml
248 mAb 1/50

FIG. 20

CD28-mediated IL-2 Pro... ...n and IL-2 Production Stimulated by CD80-Ig αtp, CD86-Ig αtp or anti-CD3





Compounds were inc... ...PCD28.1.S2.1 cells and soluble CD80Igαtp or CD86Igαtp (1 ug/ml) or anti-mous... ...ml, immobilized) for 24 hours. IL2 in the supernatants was measured by standa... ...using an IL2 dependent cell line (CTLL-20). β-galactosidase in the PCD28 cell pell... ...rofiter plates was measured using a fluorogenic substrate.

FIG. 21

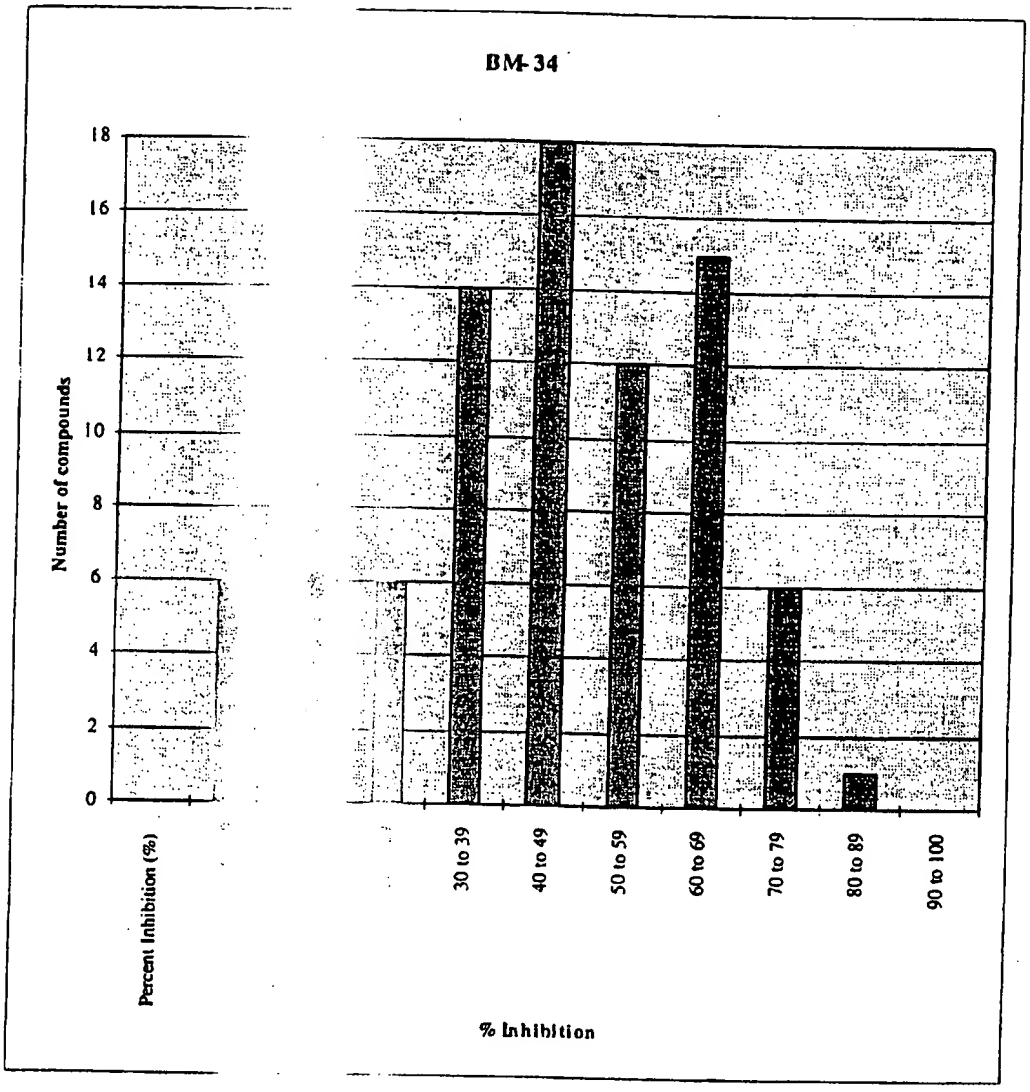
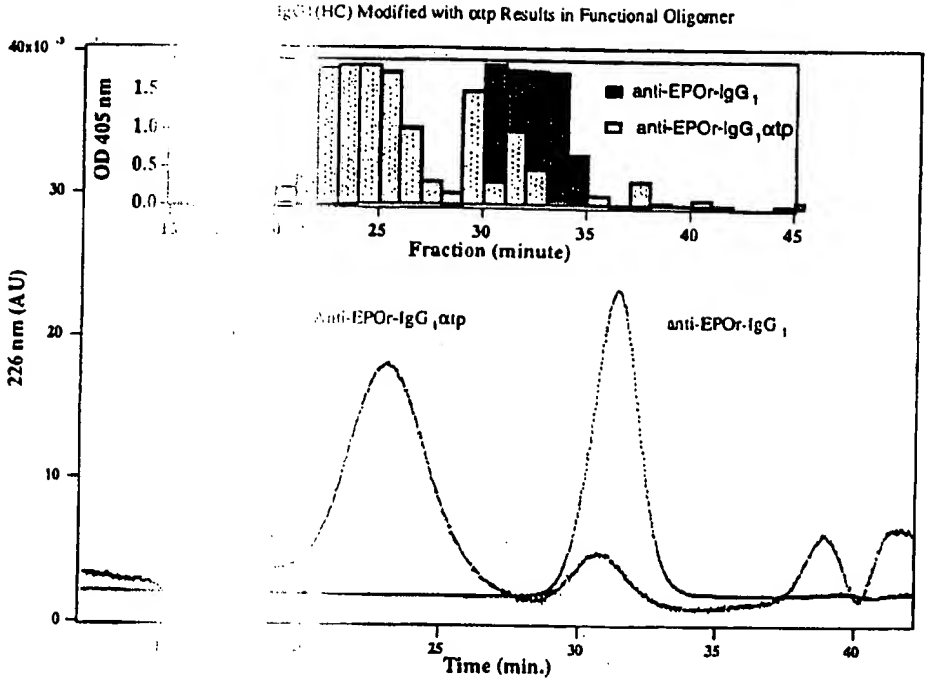

BM-34
Number of compounds
0
2
4
6
8
10
12
14
16
18
Percent Inhibition (%)
30 to 39
40 to 49
50 to 59
60 to 69
70 to 79
80 to 89
90 to 100
% Inhibition

FIG. 22


(HC) Modified with αtp Results in Functional Oligomer
OD 405 nm
anti-EPOr-IgG1
anti-EPOr-IgG1αtp
Fraction (minute)
226 nm (AU)
Anti-EPOr-IgG1αtp
anti-EPOr-IgG1
Time (min.)